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(54) Title: HUMAN CYTOKINE RECEPTOR

(57) Abstract: Cytokines and their receptors have proven usefulness in both basic research and as therapeutics. The present invention provides a new human cytokine receptor designated as "Zcytor18".

HUMAN CYTOKINE RECEPTOR

TECHNICAL FIELD

The present invention relates generally to a new protein expressed by human cells. In particular, the present invention relates to a novel gene that encodes a receptor, designated as "Zcytor18," and to nucleic acid molecules encoding Zcytor18 polypeptides.

BACKGROUND OF THE INVENTION

Cytokines are soluble, small proteins that mediate a variety of biological effects, including the regulation of the growth and differentiation of many cell types (see, for example, Arai *et al., Annu. Rev. Biochem. 59*:783 (1990); Mosmann, *Curr. Opin. Immunol. 3*:311 (1991); Paul and Seder, *Cell 76*:241 (1994)). Proteins that constitute the cytokine group include interleukins, interferons, colony stimulating factors, tumor necrosis factors, and other regulatory molecules. For example, human interleukin-17 is a cytokine which stimulates the expression of interleukin-6, intracellular adhesion molecule 1, interleukin-8, granulocyte macrophage colony-stimulating factor, and prostaglandin E2 expression, and plays a role in the preferential maturation of CD34+ hematopoietic precursors into neutrophils (Yao *et al., J. Immunol. 155*:5483 (1995); Fossiez *et al., J. Exp. Med. 183*:2593 (1996)).

Receptors that bind cytokines are typically composed of one or more integral membrane proteins that bind the cytokine with high affinity and transduce this binding event to the cell through the cytoplasmic portions of the certain receptor subunits. Cytokine receptors have been grouped into several classes on the basis of similarities in their extracellular ligand binding domains. For example, the receptor chains responsible for binding and/or transducing the effect of interferons are members of the type II cytokine receptor family, based upon a characteristic 200 residue extracellular domain.

The demonstrated *in vivo* activities of cytokines and their receptors illustrate the clinical potential of, and need for, other cytokines, cytokine receptors, cytokine agonists, and cytokine antagonists.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a novel receptor, designated "Zcytor18." The present invention also provides Zcytor18 polypeptides and Zcytor18 fusion proteins, as well as nucleic acid molecules encoding such polypeptides and proteins, and methods for using these nucleic acid molecules and amino acid sequences.

DETAILED DESCRIPTION OF THE INVENTION

1. Overview

An illustrative nucleotide sequence that encodes Zcytor18 is provided by SEQ ID NO:1. The encoded polypeptide has the following amino acid sequence: MAPWLQLCSV FFTVNACLNG SQLAVAAGGS GRARGADTCG WRMKAAARPR LCVANEGVGP ASRNSGLYNI TFKYDNCTTY LNPVGKHVIA DAQNITISQY ACHDQVAVTI LWSPGALGIE FLKGFRVILE ELKSEGRQCQ QLILKDPKQL NSSFKRTGME SQPFLNMKFE TDYFVKVVPF PSIKNESNYH PFFFRTRACD LLLQPDNLAC KPFWKPRNLN ISQHGSDMQV SFDHAPHNFG FRFFYLHYKL KHEGPFKRKT CKQEQTTETT SCLLQNVSPG DYIIELVDDT NTTRKVMHYA LKPVHSPWAG PIRAVAITVP LVVISAFATL FTVMCRKKQQ ENIYSHLDEE SSESSTYTAA LPRERLRPRP KVFLCYSSKD GQNHMNVVQC FAYFLQDFCG CEVALDLWED FSLCREGQRE WVIQKIHESQ FIIVVCSKGM KYFVDKKNYK HKGGGRGSGK GELFLVAVSA IAEKLRQAKQ SSSAALSKFI AVYFDYSCEG DVPGILDLST KYRLMDNLPQ LCSHLHSRDH GLQEPGQHTR QGSRRNYFRS KSGRSLYVAI CNMHQFIDEE PDWFEKQFVP FHPPPLRYRE PVLEKFDSGL VLNDVMCKPG PESDFCLKVE AAVLGATGPA DSQHESQHGG LDQDGEARPA LDGSAALQPL LHTVKAGSPS DMPRDSGIYD SSVPSSELSL PLMEGLSTDQ TETSSLTESV SSSSGLGEEE PPALPSKLLS SGSCKADLGC RSYTDELHAV APL (SEQ ID NO:2).

Features of the Zcytor18 polypeptide include an extracellular domain at amino acid residues 1 to 313 of SEQ ID NO:2, a putative signal sequence at amino acid residues 1 to 35 of SEQ ID NO:2, a transmembrane domain at amino acid residues 314 to 335 of SEQ ID NO:2, and an intracellular domain at amino acid residues 336 to 753 of SEQ ID NO:2. The *Zcytor18* gene resides in human chromosome 3p14.3.

Northern analysis revealed that the *Zcytor18* gene is strongly expressed in testicular, ovarian, and uterine tissue, and moderately expressed in fetal heart, fetal kidney, fetal skin, and adult brain. In contrast, little expression was detected in muscle, bladder, adult kidney, adult lung, fetal small intestine, salivary gland, or adrenal gland,

and expression was not detectable in spleen, thymus, peripheral blood leukocytes, pancreas, liver, placenta, thyroid, lymph node, or bone marrow. Studies also indicate that *Zcytor18* gene expression is higher in breast tissue than in normal breast tissue. Thus, *Zcytor18* nucleotide and amino acid sequences can be used to differentiate tissues.

One variant form of Zcytor18 is characterized by the following amino acid substitutions in the amino acid sequence of SEQ ID NO:2: Thr^{269} to Met^{269}, and Val^{750} to Ala^{750}. Additional variants of human Zcytor18 can be identified by comparison with the murine sequence (SEQ ID NO:12), such as Leu^{246} to Val^{246}, and Lys^{257} to Arg^{257}. Nucleotide, amino acid, and degenerate sequences of the human variant form are provided as SEQ ID NOs:4, 5, and 6, respectively.

A splice variant of Zcytor18 lacks amino acid residues 43 to 56 of SEQ ID NO:2. Nucleotide, amino acid, and degenerate sequences of the variant form are provided as SEQ ID NOs:7, 8, and 9, respectively. Features of this Zcytor18 splice variant include an extracellular domain at amino acid residues 1 to 299 of SEQ ID NO:8, a putative signal sequence at amino acid residues 1 to 35 of SEQ ID NO:8, a transmembrane domain at amino acid residues 300 to 321 of SEQ ID NO:8, and an intracellular domain at amino acid residues 322 to 739 of SEQ ID NO:8.

As described below, the present invention provides isolated polypeptides comprising an amino acid sequence that is at least 70%, at least 80%, or at least 90% identical to a reference amino acid sequence selected from the group consisting of: (a) an amino acid sequence comprising amino acid residues 36 to 189 of SEQ ID NO:2, (b) amino acid residues 1 to 189 of SEQ ID NO:2, (c) amino acid residues 36 to 313 of SEQ ID NO:2, (d) amino acid residues 336 to 753 of SEQ ID NO:2, (e) amino acid residues 36 to 753 of SEQ ID NO:2, (f) amino acid residues 1 to 753 of SEQ ID NO:2, (g) amino acid resides 1 to 299 of SEQ ID NO:8, (h) amino acid residues 36 to 299 of SEQ ID NO:8, (i) amino acid residues 36 to 175 of SEQ ID NO:8, (j) amino acid residues 1 to 300 of SEQ ID NO:12, (k) amino acid residues 36 to 300 of SEQ ID NO:12, and (l) 1 to 739 of SEQ ID NO:12. Certain of these polypeptides can specifically bind with an antibody that specifically binds with a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:8, or SEQ ID NO:12. Illustrative polypeptides include polypeptides comprising, or consisting of, amino acid residues 36 to 189 of SEQ ID NO:2, amino acid residues 36 to 753 of SEQ ID NO:2, 36 to 299 of SEQ ID NO:8, amino acid resides 36 to 175 of SEQ ID NO:8, or amino acid residues 36 to 300 of SEQ ID NO:12.

The present invention also provides isolated polypeptides comprising at least 15 contiguous amino acid residues of an amino acid sequence selected from the group consisting of: (a) amino acid residues 1 to 203 of SEQ ID NO:2, (b) amino acid

residues 36 to 203 of SEQ ID NO:2, (c) amino acid residues 36 to 313 of SEQ ID NO:2, (d) amino acid residues 1 to 753 of SEQ ID NO:2, (e) amino acid residues 1 to 189 of SEQ ID NO:8, (f) amino acid residues 36 to 189 of SEQ ID NO:8, (g) amino acid residues 36 to 299 of SEQ ID NO:8, (h) amino acid residues 1 to 739 of SEQ ID NO:8, (i) amino acid residues 1 to 300 of SEQ ID NO:12, (j) amino acid residues 36 to 300 of SEQ ID NO:12, and (k) 1 to 739 of SEQ ID NO:12. The present invention further provides isolated polypeptides comprising at least 30 contiguous amino acid residues of an amino acid sequence selected from the group consisting of: (l) amino acid residues 1 to 218 of SEQ ID NO:2, (m) amino acid residues 36 to 218 of SEQ ID NO:2, (n) amino acid residues 36 to 313 of SEQ ID NO:2, (o) amino acid residues 1 to 753 of SEQ ID NO:2, (p) amino acid residues 1 to 204 of SEQ ID NO:8, (q) amino acid residues 36 to 204 of SEQ ID NO:8, (r) amino acid residues 36 to 299 of SEQ ID NO:8, (s) amino acid residues 1 to 739 of SEQ ID NO:8, (t) amino acid residues 1 to 300 of SEQ ID NO:12, (u) amino acid residues 36 to 300 of SEQ ID NO:12, and (v) 1 to 739 of SEQ ID NO:12. Illustrative polypeptides include polypeptides that either comprise, or consist of, amino acid residues (a) to (v).

The present invention also includes variant Zcytor18 polypeptides, wherein the amino acid sequence of the variant polypeptide shares an identity with amino acid residues 36 to 189 of SEQ ID NO:2, amino acid residues 36 to 175 of SEQ ID NO:8, or amino acid residues 36 to 300 of SEQ ID NO:12, selected from the group consisting of at least 70% identity, at least 80% identity, at least 90% identity, at least 95% identity, or greater than 95% identity, and wherein any difference between the amino acid sequence of the variant polypeptide and the corresponding amino acid sequence of SEQ ID NO:2, SEQ ID NO:8, or SEQ ID NO:12, is due to one or more conservative amino acid substitutions. In addition, the present invention includes variant Zcytor18 polypeptides, characterized by the following amino acid substitutions in the amino acid sequence of SEQ ID NO:2: Thr^{269} to Met^{269}, and Val^{750} to Ala^{750}.

The polypeptides described herein can further comprise an affinity tag.

The present invention further provides antibodies and antibody fragments that specifically bind with such polypeptides. Exemplary antibodies include polyclonal antibodies, murine monoclonal antibodies, humanized antibodies derived from murine monoclonal antibodies, and human monoclonal antibodies. Illustrative antibody fragments include $F(ab')_2$, $F(ab)_2$, Fab', Fab, Fv, scFv, and minimal recognition units. The present invention further includes compositions comprising a carrier and a peptide, polypeptide, or antibody described herein.

The present invention also provides isolated nucleic acid molecules that encode a Zcytor18 polypeptide, wherein the nucleic acid molecule is selected from the

group consisting of: (a) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:3 or the nucleotide sequence of SEQ ID NO:9, (b) a nucleic acid molecule encoding an amino acid sequence that comprises amino acid residues 36 to 189 of SEQ ID NO:2, amino acid residues 36 to 313 of SEQ ID NO:2, amino acid residues 36 to 175 of SEQ ID NO:8, or amino acid residues 36 to 299 of SEQ ID NO:8, and (c) a nucleic acid molecule that remains hybridized following stringent wash conditions to a nucleic acid molecule consisting of the nucleotide sequence of nucleotides 192 to 652 of SEQ ID NO:1, the nucleotide sequence of nucleotides 192 to 610 of SEQ ID NO:7, the complement of the nucleotide sequence of nucleotides 192 to 652 of SEQ ID NO:1, or the complement of the nucleotide sequence of nucleotides 192 to 610 of SEQ ID NO:7. Illustrative nucleic acid molecules include those in which any difference between the amino acid sequence encoded by nucleic acid molecule (c) and the corresponding amino acid sequence of SEQ ID NO:2, or SEQ ID NO:8, is due to a conservative amino acid substitution. The present invention further contemplates isolated nucleic acid molecules that comprise nucleotides 192 to 652 of SEQ ID NO:1 or nucleotides 192 to 610 of SEQ ID NO:7, as well as nucleic acid molecules that comprise nucleotides 206 to 1000 of SEQ ID NO:11.

The present invention also includes vectors and expression vectors comprising such nucleic acid molecules. Such expression vectors may comprise a transcription promoter, and a transcription terminator, wherein the promoter is operably linked with the nucleic acid molecule, and wherein the nucleic acid molecule is operably linked with the transcription terminator. The present invention further includes recombinant host cells and recombinant viruses comprising these vectors and expression vectors. Illustrative host cells include avian, bacterial, yeast, fungal, insect, mammalian, and plant cells. Recombinant host cells comprising such expression vectors can be used to produce Zcytor18 polypeptides by culturing such recombinant host cells that comprise the expression vector and that produce the Zcytor18 protein, and, optionally, isolating the Zcytor18 protein from the cultured recombinant host cells. The present invention includes the protein products of such processes.

In addition, the present invention provides pharmaceutical compositions comprising a pharmaceutically acceptable carrier and at least one of such an expression vector or recombinant virus comprising such expression vectors. The present invention further includes pharmaceutical compositions, comprising a pharmaceutically acceptable carrier and a polypeptide described herein.

The present invention also contemplates methods for detecting the presence of *Zcytor18* RNA in a biological sample, comprising the steps of (a) contacting a *Zcytor18* nucleic acid probe under hybridizing conditions with either (i) test RNA

molecules isolated from the biological sample, or (ii) nucleic acid molecules synthesized from the isolated RNA molecules, wherein the probe has a nucleotide sequence comprising a portion of the nucleotide sequence of SEQ ID NO:1, or its complement, and (b) detecting the formation of hybrids of the nucleic acid probe and either the test RNA molecules or the synthesized nucleic acid molecules, wherein the presence of the hybrids indicates the presence of *Zcytor18* RNA in the biological sample. For example, suitable probes consist of the following nucleotide sequences: nucleotides 86 to 652 of SEQ ID NO:1, nucleotides 192 to 652 of SEQ ID NO:1, nucleotides 86 to 1024 of SEQ ID NO:1, nucleotides 192 to 1024 of SEQ ID NO:1, nucleotides 86 to 610 of SEQ ID NO:7, nucleotides 192 to 610 of SEQ ID NO:7, nucleotides 86 to 982 of SEQ ID NO:7, and nucleotides 192 to 982 of SEQ ID NO:7. Other suitable probes consist of the complement of these nucleotide sequences, or a portion of the nucleotide sequences or their complements. An example of a biological sample is a human biological sample, such as a biopsy or autopsy specimen.

The present invention further provides methods for detecting the presence of Zcytor18 polypeptide in a biological sample, comprising the steps of: (a) contacting the biological sample with an antibody or an antibody fragment that specifically binds with a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, or SEQ ID NO:8, wherein the contacting is performed under conditions that allow the binding of the antibody or antibody fragment to the biological sample, and (b) detecting any of the bound antibody or bound antibody fragment. Such an antibody or antibody fragment may further comprise a detectable label selected from the group consisting of radioisotope, fluorescent label, chemiluminescent label, enzyme label, bioluminescent label, and colloidal gold. An example of a biological sample is a human biological sample, such as a biopsy or autopsy specimen.

The present invention also provides kits for performing these detection methods. For example, a kit for detection of *Zcytor18* gene expression may comprise a container that comprises a nucleic acid molecule, wherein the nucleic acid molecule is selected from the group consisting of (a) a nucleic acid molecule comprising the nucleotide sequence of nucleotides 86 to 652 of SEQ ID NO:1, (b) a nucleic acid molecule comprising the complement of nucleotides 192 to 652 of the nucleotide sequence of SEQ ID NO:1, (c) a nucleic acid molecule comprising the nucleotide sequence of nucleotides 86 to 610 of SEQ ID NO:7, (d) a nucleic acid molecule comprising the complement of nucleotides 192 to 610 of the nucleotide sequence of SEQ ID NO:7, and (e) a nucleic acid molecule that is a fragment of (a)-(d) consisting of at least eight nucleotides. Such a kit may also comprise a second container that comprises one or more reagents capable of indicating the presence of the nucleic acid molecule. On

the other hand, a kit for detection of Zcytor18 protein may comprise a container that comprises an antibody, or an antibody fragment, that specifically binds with a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:8.

The present invention also contemplates anti-idiotype antibodies, or anti-idiotype antibody fragments, that specifically bind an antibody or antibody fragment that specifically binds a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:8. An exemplary anti-idiotype antibody binds with an antibody that specifically binds a polypeptide consisting of amino acid residues 36 to 313 of SEQ ID NO:2, amino acid residues 36 to 189 of SEQ ID NO:2, amino acid residues 36 to 299 of SEQ ID NO:8, or amino acid residues 36 to 175 of SEQ ID NO:8.

The present invention also provides isolated nucleic acid molecules comprising a nucleotide sequence that encodes a Zcytor18 secretion signal sequence and a nucleotide sequence that encodes a biologically active polypeptide, wherein the Zcytor18 secretion signal sequence comprises an amino acid sequence of residues 1 to 35 of SEQ ID NO:2. Illustrative biologically active polypeptides include Factor VIIa, proinsulin, insulin, follicle stimulating hormone, tissue type plasminogen activator, tumor necrosis factor, interleukin, colony stimulating factor, interferon, erythropoietin, and thrombopoietin. Moreover, the present invention provides fusion proteins comprising a Zcytor18 secretion signal sequence and a polypeptide, wherein the Zcytor18 secretion signal sequence comprises an amino acid sequence of residues 1 to 35 of SEQ ID NO:2.

The present invention also provides fusion proteins, comprising a Zcytor18 polypeptide and an immunoglobulin moiety. In such fusion proteins, the immunoglobulin moiety may be an immunoglobulin heavy chain constant region, such as a human F_C fragment. The present invention further includes isolated nucleic acid molecules that encode such fusion proteins.

These and other aspects of the invention will become evident upon reference to the following detailed description. In addition, various references are identified below.

2. Definitions

In the description that follows, a number of terms are used extensively. The following definitions are provided to facilitate understanding of the invention.

As used herein, "nucleic acid" or "nucleic acid molecule" refers to polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA),

oligonucleotides, fragments generated by the polymerase chain reaction (PCR), and fragments generated by any of ligation, scission, endonuclease action, and exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides (such as DNA and RNA), or analogs of naturally-occurring nucleotides (*e.g.*, α-enantiomeric forms of naturally-occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups, amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like. The term "nucleic acid molecule" also includes so-called "peptide nucleic acids," which comprise naturally-occurring or modified nucleic acid bases attached to a polyamide backbone. Nucleic acids can be either single stranded or double stranded.

The term "complement of a nucleic acid molecule" refers to a nucleic acid molecule having a complementary nucleotide sequence and reverse orientation as compared to a reference nucleotide sequence. For example, the sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCAT 3'.

The term "contig" denotes a nucleic acid molecule that has a contiguous stretch of identical or complementary sequence to another nucleic acid molecule. Contiguous sequences are said to "overlap" a given stretch of a nucleic acid molecule either in their entirety or along a partial stretch of the nucleic acid molecule.

The term "degenerate nucleotide sequence" denotes a sequence of nucleotides that includes one or more degenerate codons as compared to a reference nucleic acid molecule that encodes a polypeptide. Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (*i.e.*, GAU and GAC triplets each encode Asp).

The term "structural gene" refers to a nucleic acid molecule that is transcribed into messenger RNA (mRNA), which is then translated into a sequence of amino acids characteristic of a specific polypeptide.

An "isolated nucleic acid molecule" is a nucleic acid molecule that is not integrated in the genomic DNA of an organism. For example, a DNA molecule that

encodes a growth factor that has been separated from the genomic DNA of a cell is an isolated DNA molecule. Another example of an isolated nucleic acid molecule is a chemically-synthesized nucleic acid molecule that is not integrated in the genome of an organism. A nucleic acid molecule that has been isolated from a particular species is smaller than the complete DNA molecule of a chromosome from that species.

A "nucleic acid molecule construct" is a nucleic acid molecule, either single- or double-stranded, that has been modified through human intervention to contain segments of nucleic acid combined and juxtaposed in an arrangement not existing in nature.

"Linear DNA" denotes non-circular DNA molecules having free 5' and 3' ends. Linear DNA can be prepared from closed circular DNA molecules, such as plasmids, by enzymatic digestion or physical disruption.

"Complementary DNA (cDNA)" is a single-stranded DNA molecule that is formed from an mRNA template by the enzyme reverse transcriptase. Typically, a primer complementary to portions of mRNA is employed for the initiation of reverse transcription. Those skilled in the art also use the term "cDNA" to refer to a double-stranded DNA molecule consisting of such a single-stranded DNA molecule and its complementary DNA strand. The term "cDNA" also refers to a clone of a cDNA molecule synthesized from an RNA template.

A "promoter" is a nucleotide sequence that directs the transcription of a structural gene. Typically, a promoter is located in the 5' non-coding region of a gene, proximal to the transcriptional start site of a structural gene. Sequence elements within promoters that function in the initiation of transcription are often characterized by consensus nucleotide sequences. These promoter elements include RNA polymerase binding sites, TATA sequences, CAAT sequences, differentiation-specific elements (DSEs; McGehee *et al.*, *Mol. Endocrinol. 7*:551 (1993)), cyclic AMP response elements (CREs), serum response elements (SREs; Treisman, *Seminars in Cancer Biol. 1*:47 (1990)), glucocorticoid response elements (GREs), and binding sites for other transcription factors, such as CRE/ATF (O'Reilly *et al.*, *J. Biol. Chem. 267*:19938 (1992)), AP2 (Ye *et al.*, *J. Biol. Chem. 269*:25728 (1994)), SP1, cAMP response element binding protein (CREB; Loeken, *Gene Expr. 3*:253 (1993)) and octamer factors (see, in general, Watson *et al.*, eds., *Molecular Biology of the Gene*, 4th ed. (The Benjamin/Cummings Publishing Company, Inc. 1987), and Lemaigre and Rousseau, *Biochem. J. 303*:1 (1994)). If a promoter is an inducible promoter, then the rate of transcription increases in response to an inducing agent. In contrast, the rate of transcription is not regulated by an inducing agent if the promoter is a constitutive promoter. Repressible promoters are also known.

A "core promoter" contains essential nucleotide sequences for promoter function, including the TATA box and start of transcription. By this definition, a core promoter may or may not have detectable activity in the absence of specific sequences that may enhance the activity or confer tissue specific activity.

A "regulatory element" is a nucleotide sequence that modulates the activity of a core promoter. For example, a regulatory element may contain a nucleotide sequence that binds with cellular factors enabling transcription exclusively or preferentially in particular cells, tissues, or organelles. These types of regulatory elements are normally associated with genes that are expressed in a "cell-specific," "tissue-specific," or "organelle-specific" manner.

An "enhancer" is a type of regulatory element that can increase the efficiency of transcription, regardless of the distance or orientation of the enhancer relative to the start site of transcription.

"Heterologous DNA" refers to a DNA molecule, or a population of DNA molecules, that does not exist naturally within a given host cell. DNA molecules heterologous to a particular host cell may contain DNA derived from the host cell species (*i.e.*, endogenous DNA) so long as that host DNA is combined with non-host DNA (*i.e.*, exogenous DNA). For example, a DNA molecule containing a non-host DNA segment encoding a polypeptide operably linked to a host DNA segment comprising a transcription promoter is considered to be a heterologous DNA molecule. Conversely, a heterologous DNA molecule can comprise an endogenous gene operably linked with an exogenous promoter. As another illustration, a DNA molecule comprising a gene derived from a wild-type cell is considered to be heterologous DNA if that DNA molecule is introduced into a mutant cell that lacks the wild-type gene.

A "polypeptide" is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as "peptides."

A "protein" is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

A peptide or polypeptide encoded by a non-host DNA molecule is a "heterologous" peptide or polypeptide.

An "integrated genetic element" is a segment of DNA that has been incorporated into a chromosome of a host cell after that element is introduced into the

cell through human manipulation. Within the present invention, integrated genetic elements are most commonly derived from linearized plasmids that are introduced into the cells by electroporation or other techniques. Integrated genetic elements are passed from the original host cell to its progeny.

A "cloning vector" is a nucleic acid molecule, such as a plasmid, cosmid, or bacteriophage, which has the capability of replicating autonomously in a host cell. Cloning vectors typically contain one or a small number of restriction endonuclease recognition sites that allow insertion of a nucleic acid molecule in a determinable fashion without loss of an essential biological function of the vector, as well as nucleotide sequences encoding a marker gene that is suitable for use in the identification and selection of cells transformed with the cloning vector. Marker genes typically include genes that provide tetracycline resistance or ampicillin resistance.

An "expression vector" is a nucleic acid molecule encoding a gene that is expressed in a host cell. Typically, an expression vector comprises a transcription promoter, a gene, and a transcription terminator. Gene expression is usually placed under the control of a promoter, and such a gene is said to be "operably linked to" the promoter. Similarly, a regulatory element and a core promoter are operably linked if the regulatory element modulates the activity of the core promoter.

A "recombinant host" is a cell that contains a heterologous nucleic acid molecule, such as a cloning vector or expression vector. In the present context, an example of a recombinant host is a cell that produces Zcytor18 from an expression vector. In contrast, Zcytor18 can be produced by a cell that is a "natural source" of Zcytor18, and that lacks an expression vector.

"Integrative transformants" are recombinant host cells, in which heterologous DNA has become integrated into the genomic DNA of the cells.

A "fusion protein" is a hybrid protein expressed by a nucleic acid molecule comprising nucleotide sequences of at least two genes. For example, a fusion protein can comprise at least part of a Zcytor18 polypeptide fused with a polypeptide that binds an affinity matrix. Such a fusion protein provides a means to isolate large quantities of Zcytor18 using affinity chromatography.

The term "receptor" denotes a cell-associated protein that binds to a bioactive molecule termed a "ligand." This interaction mediates the effect of the ligand on the cell. Receptors can be membrane bound, cytosolic or nuclear; monomeric (*e.g.*, thyroid stimulating hormone receptor, beta-adrenergic receptor) or multimeric (*e.g.*, PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF receptor, erythropoietin receptor and IL-6 receptor). Membrane-bound receptors are characterized by a multi-domain structure comprising an extracellular ligand-binding

domain and an intracellular effector domain that is typically involved in signal transduction. In certain membrane-bound receptors, the extracellular ligand-binding domain and the intracellular effector domain are located in separate polypeptides that comprise the complete functional receptor.

In general, the binding of ligand to receptor results in a conformational change in the receptor that causes an interaction between the effector domain and other molecule(s) in the cell, which in turn leads to an alteration in the metabolism of the cell. Metabolic events that are often linked to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids.

The term "secretory signal sequence" denotes a DNA sequence that encodes a peptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

An "isolated polypeptide" is a polypeptide that is essentially free from contaminating cellular components, such as carbohydrate, lipid, or other proteinaceous impurities associated with the polypeptide in nature. Typically, a preparation of isolated polypeptide contains the polypeptide in a highly purified form, *i.e.*, at least about 80% pure, at least about 90% pure, at least about 95% pure, greater than 95% pure, or greater than 99% pure. One way to show that a particular protein preparation contains an isolated polypeptide is by the appearance of a single band following sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of the protein preparation and Coomassie Brilliant Blue staining of the gel. However, the term "isolated" does not exclude the presence of the same polypeptide in alternative physical forms, such as dimers or alternatively glycosylated or derivatized forms.

The terms "amino-terminal" and "carboxyl-terminal" are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus of the complete polypeptide.

The term "expression" refers to the biosynthesis of a gene product. For example, in the case of a structural gene, expression involves transcription of the structural gene into mRNA and the translation of mRNA into one or more polypeptides.

The term "splice variant" is used herein to denote alternative forms of RNA transcribed from a gene. Splice variation arises naturally through use of alternative splicing sites within a transcribed RNA molecule, or less commonly between separately transcribed RNA molecules, and may result in several mRNAs transcribed from the same gene. Splice variants may encode polypeptides having altered amino acid sequence. The term splice variant is also used herein to denote a polypeptide encoded by a splice variant of an mRNA transcribed from a gene.

As used herein, the term "immunomodulator" includes cytokines, stem cell growth factors, lymphotoxins, co-stimulatory molecules, hematopoietic factors, and synthetic analogs of these molecules.

The term "complement/anti-complement pair" denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the complement/anti-complement pair is desirable, the complement/anti-complement pair preferably has a binding affinity of less than 10^9 M^{-1}.

An "anti-idiotype antibody" is an antibody that binds with the variable region domain of an immunoglobulin. In the present context, an anti-idiotype antibody binds with the variable region of an anti-Zcytor18 antibody, and thus, an anti-idiotype antibody mimics an epitope of Zcytor18.

An "antibody fragment" is a portion of an antibody such as $F(ab')_2$, $F(ab)_2$, Fab', Fab, and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. For example, an anti-Zcytor18 monoclonal antibody fragment binds with an epitope of Zcytor18.

The term "antibody fragment" also includes a synthetic or a genetically engineered polypeptide that binds to a specific antigen, such as polypeptides consisting of the light chain variable region, "Fv" fragments consisting of the variable regions of the heavy and light chains, recombinant single chain polypeptide molecules in which light and heavy variable regions are connected by a peptide linker ("scFv proteins"), and minimal recognition units consisting of the amino acid residues that mimic the hypervariable region.

A "chimeric antibody" is a recombinant protein that contains the variable domains and complementary determining regions derived from a rodent antibody, while the remainder of the antibody molecule is derived from a human antibody.

"Humanized antibodies" are recombinant proteins in which murine complementarity determining regions of a monoclonal antibody have been transferred from

heavy and light variable chains of the murine immunoglobulin into a human variable domain.

As used herein, a "therapeutic agent" is a molecule or atom, which is conjugated to an antibody moiety to produce a conjugate, which is useful for therapy. Examples of therapeutic agents include drugs, toxins, immunomodulators, chelators, boron compounds, photoactive agents or dyes, and radioisotopes.

A "detectable label" is a molecule or atom, which can be conjugated to an antibody moiety to produce a molecule useful for diagnosis. Examples of detectable labels include chelators, photoactive agents, radioisotopes, fluorescent agents, paramagnetic ions, or other marker moieties.

The term "affinity tag" is used herein to denote a polypeptide segment that can be attached to a second polypeptide to provide for purification or detection of the second polypeptide or provide sites for attachment of the second polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag. Affinity tags include a poly-histidine tract, protein A (Nilsson *et al., EMBO J. 4*:1075 (1985); Nilsson *et al., Methods Enzymol. 198*:3 (1991)), glutathione S transferase (Smith and Johnson, *Gene 67*:31 (1988)), Glu-Glu affinity tag (Grussenmeyer *et al., Proc. Natl. Acad. Sci. USA 82*:7952 (1985)), substance P, FLAG peptide (Hopp *et al., Biotechnology 6*:1204 (1988)), streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general, Ford *et al., Protein Expression and Purification* 2:95 (1991). DNA molecules encoding affinity tags are available from commercial suppliers (*e.g.*, Pharmacia Biotech, Piscataway, NJ).

A "naked antibody" is an entire antibody, as opposed to an antibody fragment, which is not conjugated with a therapeutic agent. Naked antibodies include both polyclonal and monoclonal antibodies, as well as certain recombinant antibodies, such as chimeric and humanized antibodies.

As used herein, the term "antibody component" includes both an entire antibody and an antibody fragment.

An "immunoconjugate" is a conjugate of an antibody component with a therapeutic agent or a detectable label.

As used herein, the term "antibody fusion protein" refers to a recombinant molecule that comprises an antibody component and a Zcytor18 polypeptide component. Examples of an antibody fusion protein include a protein that comprises a Zcytor18 extracellular domain, and either an Fc domain or an antigen-biding region.

A "target polypeptide" or a "target peptide" is an amino acid sequence that comprises at least one epitope, and that is expressed on a target cell, such as a tumor

cell, or a cell that carries an infectious agent antigen. T cells recognize peptide epitopes presented by a major histocompatibility complex molecule to a target polypeptide or target peptide and typically lyse the target cell or recruit other immune cells to the site of the target cell, thereby killing the target cell.

An "antigenic peptide" is a peptide, which will bind a major histocompatibility complex molecule to form an MHC-peptide complex, which is recognized by a T cell, thereby inducing a cytotoxic lymphocyte response upon presentation to the T cell. Thus, antigenic peptides are capable of binding to an appropriate major histocompatibility complex molecule and inducing a cytotoxic T cells response, such as cell lysis or specific cytokine release against the target cell, which binds or expresses the antigen. The antigenic peptide can be bound in the context of a class I or class II major histocompatibility complex molecule, on an antigen presenting cell or on a target cell.

In eukaryotes, RNA polymerase II catalyzes the transcription of a structural gene to produce mRNA. A nucleic acid molecule can be designed to contain an RNA polymerase II template in which the RNA transcript has a sequence that is complementary to that of a specific mRNA. The RNA transcript is termed an "anti-sense RNA" and a nucleic acid molecule that encodes the anti-sense RNA is termed an "anti-sense gene." Anti-sense RNA molecules are capable of binding to mRNA molecules, resulting in an inhibition of mRNA translation.

An "anti-sense oligonucleotide specific for Zcytor18" or a "Zcytor18 anti-sense oligonucleotide" is an oligonucleotide having a sequence (a) capable of forming a stable triplex with a portion of the *Zcytor18* gene, or (b) capable of forming a stable duplex with a portion of an mRNA transcript of the *Zcytor18* gene.

A "ribozyme" is a nucleic acid molecule that contains a catalytic center. The term includes RNA enzymes, self-splicing RNAs, self-cleaving RNAs, and nucleic acid molecules that perform these catalytic functions. A nucleic acid molecule that encodes a ribozyme is termed a "ribozyme gene."

An "external guide sequence" is a nucleic acid molecule that directs the endogenous ribozyme, RNase P, to a particular species of intracellular mRNA, resulting in the cleavage of the mRNA by RNase P. A nucleic acid molecule that encodes an external guide sequence is termed an "external guide sequence gene."

The term "variant *Zcytor18* gene" refers to nucleic acid molecules that encode a polypeptide having an amino acid sequence that is a modification of SEQ ID NO:2. Such variants include naturally-occurring polymorphisms of *Zcytor18* genes, as well as synthetic genes that contain conservative amino acid substitutions of the amino acid sequence of SEQ ID NO:2. Additional variant forms of *Zcytor18* genes are nucleic

acid molecules that contain insertions or deletions of the nucleotide sequences described herein. A variant *Zcytor18* gene can be identified, for example, by determining whether the gene hybridizes with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, or its complement, under stringent conditions.

Alternatively, variant *Zcytor18* genes can be identified by sequence comparison. Two amino acid sequences have "100% amino acid sequence identity" if the amino acid residues of the two amino acid sequences are the same when aligned for maximal correspondence. Similarly, two nucleotide sequences have "100% nucleotide sequence identity" if the nucleotide residues of the two nucleotide sequences are the same when aligned for maximal correspondence. Sequence comparisons can be performed using standard software programs such as those included in the LASERGENE bioinformatics computing suite, which is produced by DNASTAR (Madison, Wisconsin). Other methods for comparing two nucleotide or amino acid sequences by determining optimal alignment are well-known to those of skill in the art (see, for example, Peruski and Peruski, *The Internet and the New Biology: Tools for Genomic and Molecular Research* (ASM Press, Inc. 1997), Wu *et al.* (eds.), "Information Superhighway and Computer Databases of Nucleic Acids and Proteins," in *Methods in Gene Biotechnology*, pages 123-151 (CRC Press, Inc. 1997), and Bishop (ed.), *Guide to Human Genome Computing*, 2nd Edition (Academic Press, Inc. 1998)). Particular methods for determining sequence identity are described below.

Regardless of the particular method used to identify a variant *Zcytor18* gene or variant Zcytor18 polypeptide, a variant gene or polypeptide encoded by a variant gene may be functionally characterized the ability to bind specifically to an anti-Zcytor18 antibody.

The term "allelic variant" is used herein to denote any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene.

The term "ortholog" denotes a polypeptide or protein obtained from one species that is the functional counterpart of a polypeptide or protein from a different species. Sequence differences among orthologs are the result of speciation.

"Paralogs" are distinct but structurally related proteins made by an organism. Paralogs are believed to arise through gene duplication. For example, α-globin, β-globin, and myoglobin are paralogs of each other.

The present invention includes functional fragments of *Zcytor18* genes. Within the context of this invention, a "functional fragment" of a *Zcytor18* gene refers to a nucleic acid molecule that encodes a portion of a Zcytor18 polypeptide, which is a domain described herein or at least specifically binds with an anti-Zcytor18 antibody.

Due to the imprecision of standard analytical methods, molecular weights and lengths of polymers are understood to be approximate values. When such a value is expressed as "about" X or "approximately" X, the stated value of X will be understood to be accurate to ±10%.

3. *Production of Nucleic Acid Molecules Encoding Zcytor18*

Nucleic acid molecules encoding a human Zcytor18 can be obtained by screening a human cDNA or genomic library using polynucleotide probes based upon SEQ ID NO:1. These techniques are standard and well-established.

As an illustration, a nucleic acid molecule that encodes a human Zcytor18 can be isolated from a cDNA library. In this case, the first step would be to prepare the cDNA library by isolating RNA from a tissue, such as testicular tissue, using methods well-known to those of skill in the art. In general, RNA isolation techniques must provide a method for breaking cells, a means of inhibiting RNase-directed degradation of RNA, and a method of separating RNA from DNA, protein, and polysaccharide contaminants. For example, total RNA can be isolated by freezing tissue in liquid nitrogen, grinding the frozen tissue with a mortar and pestle to lyse the cells, extracting the ground tissue with a solution of phenol/chloroform to remove proteins, and separating RNA from the remaining impurities by selective precipitation with lithium chloride (see, for example, Ausubel *et al.* (eds.), *Short Protocols in Molecular Biology, 3rd Edition*, pages 4-1 to 4-6 (John Wiley & Sons 1995) ["Ausubel (1995)"]; Wu *et al.*, *Methods in Gene Biotechnology*, pages 33-41 (CRC Press, Inc. 1997) ["Wu (1997)"]).

Alternatively, total RNA can be isolated by extracting ground tissue with guanidinium isothiocyanate, extracting with organic solvents, and separating RNA from contaminants using differential centrifugation (see, for example, Chirgwin *et al.*, *Biochemistry 18*:52 (1979); Ausubel (1995) at pages 4-1 to 4-6; Wu (1997) at pages 33-41).

In order to construct a cDNA library, poly(A)$^+$ RNA must be isolated from a total RNA preparation. Poly(A)$^+$ RNA can be isolated from total RNA using the standard technique of oligo(dT)-cellulose chromatography (see, for example, Aviv and Leder, *Proc. Nat'l Acad. Sci. USA 69*:1408 (1972); Ausubel (1995) at pages 4-11 to 4-12).

Double-stranded cDNA molecules are synthesized from poly(A)$^+$ RNA using techniques well-known to those in the art. (see, for example, Wu (1997) at pages 41-46). Moreover, commercially available kits can be used to synthesize double-stranded cDNA molecules. For example, such kits are available from Life Technologies, Inc. (Gaithersburg, MD), CLONTECH Laboratories, Inc. (Palo Alto, CA), Promega Corporation (Madison, WI) and STRATAGENE (La Jolla, CA).

Various cloning vectors are appropriate for the construction of a cDNA library. For example, a cDNA library can be prepared in a vector derived from bacteriophage, such as a λgt10 vector. See, for example, Huynh *et al.*, "Constructing and Screening cDNA Libraries in λgt10 and λgt11," in *DNA Cloning: A Practical Approach Vol. I*, Glover (ed.), page 49 (IRL Press, 1985); Wu (1997) at pages 47-52.

Alternatively, double-stranded cDNA molecules can be inserted into a plasmid vector, such as a PBLUESCRIPT vector (STRATAGENE; La Jolla, CA), a LAMDAGEM-4 (Promega Corp.) or other commercially available vectors. Suitable cloning vectors also can be obtained from the American Type Culture Collection (Manassas, VA).

To amplify the cloned cDNA molecules, the cDNA library is inserted into a prokaryotic host, using standard techniques. For example, a cDNA library can be introduced into competent *E. coli* DH5 cells, which can be obtained, for example, from Life Technologies, Inc. (Gaithersburg, MD).

A human genomic library can be prepared by means well-known in the art (see, for example, Ausubel (1995) at pages 5-1 to 5-6; Wu (1997) at pages 307-327). Genomic DNA can be isolated by lysing tissue with the detergent Sarkosyl, digesting the lysate with proteinase K, clearing insoluble debris from the lysate by centrifugation, precipitating nucleic acid from the lysate using isopropanol, and purifying resuspended DNA on a cesium chloride density gradient.

DNA fragments that are suitable for the production of a genomic library can be obtained by the random shearing of genomic DNA or by the partial digestion of genomic DNA with restriction endonucleases. Genomic DNA fragments can be inserted into a vector, such as a bacteriophage or cosmid vector, in accordance with conventional techniques, such as the use of restriction enzyme digestion to provide appropriate termini, the use of alkaline phosphatase treatment to avoid undesirable joining of DNA molecules, and ligation with appropriate ligases. Techniques for such manipulation are well-known in the art (see, for example, Ausubel (1995) at pages 5-1 to 5-6; Wu (1997) at pages 307-327).

Alternatively, human genomic libraries can be obtained from commercial sources such as Research Genetics (Huntsville, AL) and the American Type Culture Collection (Manassas, VA).

A library containing cDNA or genomic clones can be screened with one or more polynucleotide probes based upon SEQ ID NO:1, using standard methods (see, for example, Ausubel (1995) at pages 6-1 to 6-11).

Nucleic acid molecules that encode a human *Zcytor18* gene can also be obtained using the polymerase chain reaction (PCR) with oligonucleotide primers having nucleotide sequences that are based upon the nucleotide sequences of the *Zcytor18* gene, as described herein. General methods for screening libraries with PCR are provided by, for example, Yu *et al.*, "Use of the Polymerase Chain Reaction to Screen Phage Libraries," in *Methods in Molecular Biology, Vol. 15: PCR Protocols: Current Methods and Applications*, White (ed.), pages 211-215 (Humana Press, Inc. 1993). Moreover, techniques for using PCR to isolate related genes are described by, for example, Preston, "Use of Degenerate Oligonucleotide Primers and the Polymerase Chain Reaction to Clone Gene Family Members," in *Methods in Molecular Biology, Vol. 15: PCR Protocols: Current Methods and Applications*, White (ed.), pages 317-337 (Humana Press, Inc. 1993).

Anti-Zcytor18 antibodies, produced as described below, can also be used to isolate DNA sequences that encode human *Zcytor18* genes from cDNA libraries. For example, the antibodies can be used to screen λgt11 expression libraries, or the antibodies can be used for immunoscreening following hybrid selection and translation (see, for example, Ausubel (1995) at pages 6-12 to 6-16; Margolis *et al.*, "Screening λ expression libraries with antibody and protein probes," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), pages 1-14 (Oxford University Press 1995)).

As an alternative, a *Zcytor18* gene can be obtained by synthesizing nucleic acid molecules using mutually priming long oligonucleotides and the nucleotide sequences described herein (see, for example, Ausubel (1995) at pages 8-8 to 8-9). Established techniques using the polymerase chain reaction provide the ability to synthesize DNA molecules at least two kilobases in length (Adang *et al.*, *Plant Molec. Biol. 21*:1131 (1993), Bambot *et al.*, *PCR Methods and Applications 2*:266 (1993), Dillon *et al.*, "Use of the Polymerase Chain Reaction for the Rapid Construction of Synthetic Genes," in *Methods in Molecular Biology, Vol. 15: PCR Protocols: Current Methods and Applications*, White (ed.), pages 263-268, (Humana Press, Inc. 1993), and Holowachuk *et al., PCR Methods Appl. 4*:299 (1995)).

The nucleic acid molecules of the present invention can also be synthesized with "gene machines" using protocols such as the phosphoramidite method. If chemically-synthesized double stranded DNA is required for an application such as the synthesis of a gene or a gene fragment, then each complementary strand is made separately. The production of short genes (60 to 80 base pairs) is technically

straightforward and can be accomplished by synthesizing the complementary strands and then annealing them. For the production of longer genes (>300 base pairs), however, special strategies may be required, because the coupling efficiency of each cycle during chemical DNA synthesis is seldom 100%. To overcome this problem, synthetic genes (double-stranded) are assembled in modular form from single-stranded fragments that are from 20 to 100 nucleotides in length. For reviews on polynucleotide synthesis, see, for example, Glick and Pasternak, *Molecular Biotechnology, Principles and Applications of Recombinant DNA* (ASM Press 1994), Itakura *et al., Annu. Rev. Biochem. 53*:323 (1984), and Climie *et al., Proc. Nat'l Acad. Sci. USA 87*:633 (1990).

The sequence of a *Zcytor18* cDNA or *Zcytor18* genomic fragment can be determined using standard methods. Zcytor18 polynucleotide sequences disclosed herein can also be used as probes or primers to clone 5' non-coding regions of a *Zcytor18* gene. Promoter elements from a *Zcytor18* gene can be used to direct the expression of heterologous genes in, for example, transgenic animals or patients treated with gene therapy. The identification of genomic fragments containing a *Zcytor18* promoter or regulatory element can be achieved using well-established techniques, such as deletion analysis (see, generally, Ausubel (1995)).

Cloning of 5' flanking sequences also facilitates production of Zcytor18 proteins by "gene activation," as disclosed in U.S. Patent No. 5,641,670. Briefly, expression of an endogenous *Zcytor18* gene in a cell is altered by introducing into the *Zcytor18* locus a DNA construct comprising at least a targeting sequence, a regulatory sequence, an exon, and an unpaired splice donor site. The targeting sequence is a *Zcytor18* 5' non-coding sequence that permits homologous recombination of the construct with the endogenous *Zcytor18* locus, whereby the sequences within the construct become operably linked with the endogenous *Zcytor18* coding sequence. In this way, an endogenous *Zcytor18* promoter can be replaced or supplemented with other regulatory sequences to provide enhanced, tissue-specific, or otherwise regulated expression.

4. *Production of Zcytor18 Variants*

The present invention provides a variety of nucleic acid molecules, including DNA and RNA molecules, which encode the Zcytor18 polypeptides disclosed herein. Those skilled in the art will readily recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules. SEQ ID NO:3 is a degenerate nucleotide sequence that encompasses all nucleic acid molecules that encode the Zcytor18 polypeptide of SEQ ID NO:2. Those

skilled in the art will recognize that the degenerate sequence of SEQ ID NO:3 also provides all RNA sequences encoding SEQ ID NO:2, by substituting U for T. Thus, the present invention contemplates Zcytor18 polypeptide-encoding nucleic acid molecules comprising nucleotide 86 to nucleotide 2344 of SEQ ID NO:1, and their RNA equivalents. Similarly, the present invention contemplates Zcytor18 polypeptide-encoding nucleic acid molecules comprising nucleotide 86 to nucleotide 2302 of SEQ ID NO:7, and their RNA equivalents.

Table 1 sets forth the one-letter codes used within SEQ ID NO:3 to denote degenerate nucleotide positions. "Resolutions" are the nucleotides denoted by a code letter. "Complement" indicates the code for the complementary nucleotide(s). For example, the code Y denotes either C or T, and its complement R denotes A or G, A being complementary to T, and G being complementary to C.

Table 1

Nucleotide	Resolution	Complement	Resolution
A	A	T	T
C	C	G	G
G	G	C	C
T	T	A	A
R	A|G	Y	C|T
Y	C|T	R	A|G
M	A|C	K	G|T
K	G|T	M	A|C
S	C|G	S	C|G
W	A|T	W	A|T
H	A|C|T	D	A|G|T
B	C|G|T	V	A|C|G
V	A|C|G	B	C|G|T
D	A|G|T	H	A|C|T
N	A|C|G|T	N	A|C|G|T

The degenerate codons used in SEQ ID NO:3, encompassing all possible codons for a given amino acid, are set forth in Table 2.

Table 2

Amino Acid	One Letter Code	Codons	Degenerate Codon
Cys	C	TGC TGT	TGY
Ser	S	AGC AGT TCA TCC TCG TCT	WSN
Thr	T	ACA ACC ACG ACT	ACN
Pro	P	CCA CCC CCG CCT	CCN
Ala	A	GCA GCC GCG GCT	GCN
Gly	G	GGA GGC GGG GGT	GGN
Asn	N	AAC AAT	AAY
Asp	D	GAC GAT	GAY
Glu	E	GAA GAG	GAR
Gln	Q	CAA CAG	CAR
His	H	CAC CAT	CAY
Arg	R	AGA AGG CGA CGC CGG CGT	MGN
Lys	K	AAA AAG	AAR
Met	M	ATG	ATG
Ile	I	ATA ATC ATT	ATH
Leu	L	CTA CTC CTG CTT TTA TTG	YTN
Val	V	GTA GTC GTG GTT	GTN
Phe	F	TTC TTT	TTY
Tyr	Y	TAC TAT	TAY
Trp	W	TGG	TGG
Ter	.	TAA TAG TGA	TRR
Asn|Asp	B		RAY
Glu|Gln	Z		SAR
Any	X		NNN

One of ordinary skill in the art will appreciate that some ambiguity is introduced in determining a degenerate codon, representative of all possible codons encoding an amino acid. For example, the degenerate codon for serine (WSN) can, in some circumstances, encode arginine (AGR), and the degenerate codon for arginine (MGN) can, in some circumstances, encode serine (AGY). A similar relationship exists between codons encoding phenylalanine and leucine. Thus, some polynucleotides encompassed by the degenerate sequence may encode variant amino acid sequences, but one of ordinary skill in the art can easily identify such variant sequences by reference to the amino acid sequences of SEQ ID NO:2. Variant sequences can be readily tested for functionality as described herein.

Different species can exhibit "preferential codon usage." In general, see, Grantham *et al., Nucl. Acids Res. 8*:1893 (1980), Haas *et al. Curr. Biol. 6*:315 (1996), Wain-Hobson *et al., Gene 13*:355 (1981), Grosjean and Fiers, *Gene 18*:199 (1982), Holm, *Nuc. Acids Res. 14*:3075 (1986), Ikemura, *J. Mol. Biol. 158*:573 (1982), Sharp and Matassi, *Curr. Opin. Genet. Dev. 4*:851 (1994), Kane, *Curr. Opin. Biotechnol. 6*:494 (1995), and Makrides, *Microbiol. Rev. 60*:512 (1996). As used herein, the term "preferential codon usage" or "preferential codons" is a term of art referring to protein translation codons that are most frequently used in cells of a certain species, thus favoring one or a few representatives of the possible codons encoding each amino acid (See Table 2). For example, the amino acid threonine (thr) may be encoded by ACA, ACC, ACG, or ACT, but in mammalian cells ACC is the most commonly used codon; in other species, for example, insect cells, yeast, viruses or bacteria, different threonine codons may be preferential. Preferential codons for a particular species can be introduced into the polynucleotides of the present invention by a variety of methods known in the art. Introduction of preferential codon sequences into recombinant DNA can, for example, enhance production of the protein by making protein translation more efficient within a particular cell type or species. Therefore, the degenerate codon sequences disclosed herein serve as a template for optimizing expression of polynucleotides in various cell types and species commonly used in the art and disclosed herein. Sequences containing preferential codons can be tested and optimized for expression in various species, and tested for functionality as disclosed herein.

The present invention further provides variant polypeptides and nucleic acid molecules that represent counterparts from other species (orthologs). These species include, but are not limited to mammalian, avian, amphibian, reptile, fish, insect and other vertebrate and invertebrate species. As an illustration, SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:13 provide the nucleotide, amino acid, and degenerate nucleotide sequences, respectively, of murine Zcytor18. Features of the murine Zcytor18

polypeptide include an extracellular domain at amino acid residues 1 to 300 of SEQ ID NO:12, a putative signal sequence at amino acid residues 1 to 35 of SEQ ID NO:12, a transmembrane domain at amino acid residues 301 to 322 of SEQ ID NO:12, and an intracellular domain at amino acid residues 323 to 739 of SEQ ID NO:12. Murine *Zcytor18* gene expression has been detected in brain, kidney, lung, skin, testis, and uterus tissues of the mouse, while little or no expression was detectable in heart, liver, pancreas, and spleen tissues.

Zcytor18 polypeptides from other mammalian species, including mouse, porcine, ovine, bovine, canine, feline, equine, and other primate polypeptides, are also of interest. Orthologs of human Zcytor18 can be cloned using information and compositions provided by the present invention in combination with conventional cloning techniques. For example, a *Zcytor18* cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses *Zcytor18* as disclosed herein. Suitable sources of mRNA can be identified by probing northern blots with probes designed from the sequences disclosed herein. A library is then prepared from mRNA of a positive tissue or cell line.

A *Zcytor18*-encoding cDNA can be isolated by a variety of methods, such as by probing with a complete or partial human cDNA or with one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also be cloned using the polymerase chain reaction with primers designed from the representative human *Zcytor18* sequences disclosed herein. In addition, a cDNA library can be used to transform or transfect host cells, and expression of the cDNA of interest can be detected with an antibody to Zcytor18 polypeptide.

Those skilled in the art will recognize that the sequence disclosed in SEQ ID NO:1 represents a single allele of human *Zcytor18*, and that allelic variation and alternative splicing are expected to occur. Allelic variants of this sequence can be cloned by probing cDNA or genomic libraries from different individuals according to standard procedures. Allelic variants of the nucleotide sequences disclosed herein, including those containing silent mutations and those in which mutations result in amino acid sequence changes, are within the scope of the present invention, as are proteins which are allelic variants of the amino acid sequences disclosed herein. cDNA molecules generated from alternatively spliced mRNAs, which retain the properties of the Zcytor18 polypeptide are included within the scope of the present invention, as are polypeptides encoded by such cDNAs and mRNAs. Allelic variants and splice variants of these sequences can be cloned by probing cDNA or genomic libraries from different individuals or tissues according to standard procedures known in the art.

Within certain embodiments of the invention, the isolated nucleic acid molecules can hybridize under stringent conditions to nucleic acid molecules comprising nucleotide sequences disclosed herein. For example, such nucleic acid molecules can hybridize under stringent conditions to nucleic acid molecules comprising the nucleotide sequence of SEQ ID NO:1, to nucleic acid molecules consisting of the nucleotide sequence of nucleotides 192 to 652 of SEQ ID NO:1, nucleotide sequence of nucleotides 192 to 2344 of SEQ ID NO:1, or to nucleic acid molecules comprising a nucleotide sequence complementary to SEQ ID NO:1, the nucleotide sequence of nucleotides 192 to 652 of SEQ ID NO:1, or nucleotides 192 to 2344 of SEQ ID NO:1. In general, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe.

A pair of nucleic acid molecules, such as DNA-DNA, RNA-RNA and DNA-RNA, can hybridize if the nucleotide sequences have some degree of complementarity. Hybrids can tolerate mismatched base pairs in the double helix, but the stability of the hybrid is influenced by the degree of mismatch. The T_m of the mismatched hybrid decreases by 1°C for every 1-1.5% base pair mismatch. Varying the stringency of the hybridization conditions allows control over the degree of mismatch that will be present in the hybrid. The degree of stringency increases as the hybridization temperature increases and the ionic strength of the hybridization buffer decreases. Stringent hybridization conditions encompass temperatures of about 5-25°C below the T_m of the hybrid and a hybridization buffer having up to 1 M Na^+. Higher degrees of stringency at lower temperatures can be achieved with the addition of formamide which reduces the T_m of the hybrid about 1°C for each 1% formamide in the buffer solution. Generally, such stringent conditions include temperatures of 20-70°C and a hybridization buffer containing up to 6x SSC and 0-50% formamide. A higher degree of stringency can be achieved at temperatures of from 40-70°C with a hybridization buffer having up to 4x SSC and from 0-50% formamide. Highly stringent conditions typically encompass temperatures of 42-70°C with a hybridization buffer having up to 1x SSC and 0-50% formamide. Different degrees of stringency can be used during hybridization and washing to achieve maximum specific binding to the target sequence. Typically, the washes following hybridization are performed at increasing degrees of stringency to remove non-hybridized polynucleotide probes from hybridized complexes.

The above conditions are meant to serve as a guide and it is well within the abilities of one skilled in the art to adapt these conditions for use with a particular polypeptide hybrid. The T_m for a specific target sequence is the temperature (under

defined conditions) at which 50% of the target sequence will hybridize to a perfectly matched probe sequence. Conditions that influence the T_m include, the size and base pair content of the polynucleotide probe, the ionic strength of the hybridization solution, and the presence of destabilizing agents in the hybridization solution. Numerous equations for calculating T_m are known in the art, and are specific for DNA, RNA and DNA-RNA hybrids and polynucleotide probe sequences of varying length (see, for example, Sambrook *et al., Molecular Cloning: A Laboratory Manual*, Second Edition (Cold Spring Harbor Press 1989); Ausubel *et al.*, (eds.), *Current Protocols in Molecular Biology* (John Wiley and Sons, Inc. 1987); Berger and Kimmel (eds.), *Guide to Molecular Cloning Techniques*, (Academic Press, Inc. 1987); and Wetmur, *Crit. Rev. Biochem. Mol. Biol. 26*:227 (1990)). Sequence analysis software such as OLIGO 6.0 (LSR; Long Lake, MN) and *Primer Premier 4.0* (Premier Biosoft International; Palo Alto, CA), as well as sites on the Internet, are available tools for analyzing a given sequence and calculating T_m based on user defined criteria. Such programs can also analyze a given sequence under defined conditions and identify suitable probe sequences. Typically, hybridization of longer polynucleotide sequences, >50 base pairs, is performed at temperatures of about 20-25°C below the calculated T_m. For smaller probes, <50 base pairs, hybridization is typically carried out at the T_m or 5-10°C below. This allows for the maximum rate of hybridization for DNA-DNA and DNA-RNA hybrids.

The length of the polynucleotide sequence influences the rate and stability of hybrid formation. Smaller probe sequences, <50 base pairs, reach equilibrium with complementary sequences rapidly, but may form less stable hybrids. Incubation times of anywhere from minutes to hours can be used to achieve hybrid formation. Longer probe sequences come to equilibrium more slowly, but form more stable complexes even at lower temperatures. Incubations are allowed to proceed overnight or longer. Generally, incubations are carried out for a period equal to three times the calculated Cot time. Cot time, the time it takes for the polynucleotide sequences to reassociate, can be calculated for a particular sequence by methods known in the art.

The base pair composition of polynucleotide sequence will effect the thermal stability of the hybrid complex, thereby influencing the choice of hybridization temperature and the ionic strength of the hybridization buffer. A-T pairs are less stable than G-C pairs in aqueous solutions containing sodium chloride. Therefore, the higher the G-C content, the more stable the hybrid. Even distribution of G and C residues within the sequence also contribute positively to hybrid stability. In addition, the base pair composition can be manipulated to alter the T_m of a given sequence. For example, 5-methyldeoxycytidine can be substituted for deoxycytidine and 5-bromodeoxuridine can

be substituted for thymidine to increase the T_m, whereas 7-deazz-2'-deoxyguanosine can be substituted for guanosine to reduce dependence on T_m.

The ionic concentration of the hybridization buffer also affects the stability of the hybrid. Hybridization buffers generally contain blocking agents such as Denhardt's solution (Sigma Chemical Co., St. Louis, Mo.), denatured salmon sperm DNA, tRNA, milk powders (BLOTTO), heparin or SDS, and a Na^+ source, such as SSC (1x SSC: 0.15 M sodium chloride, 15 mM sodium citrate) or SSPE (1x SSPE: 1.8 M NaCl, 10 mM NaH_2PO_4, 1 mM EDTA, pH 7.7). Typically, hybridization buffers contain from between 10 mM - 1 M Na^+. The addition of destabilizing or denaturing agents such as formamide, tetralkylammonium salts, guanidinium cations or thiocyanate cations to the hybridization solution will alter the T_m of a hybrid. Typically, formamide is used at a concentration of up to 50% to allow incubations to be carried out at more convenient and lower temperatures. Formamide also acts to reduce non-specific background when using RNA probes.

As an illustration, a nucleic acid molecule encoding a variant Zcytor18 polypeptide can be hybridized with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 (or its complement) at 42°C overnight in a solution comprising 50% formamide, 5x SSC, 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution (100x Denhardt's solution: 2% (w/v) Ficoll 400, 2% (w/v) polyvinylpyrrolidone, and 2% (w/v) bovine serum albumin), 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA. One of skill in the art can devise variations of these hybridization conditions. For example, the hybridization mixture can be incubated at a higher temperature, such as about 65°C, in a solution that does not contain formamide. Moreover, premixed hybridization solutions are available (*e.g.*, EXPRESSHYB Hybridization Solution from CLONTECH Laboratories, Inc.), and hybridization can be performed according to the manufacturer's instructions.

Following hybridization, the nucleic acid molecules can be washed to remove non-hybridized nucleic acid molecules under stringent conditions, or under highly stringent conditions. Typical stringent washing conditions include washing in a solution of 0.5x - 2x SSC with 0.1% sodium dodecyl sulfate (SDS) at 55 - 65°C. As an illustration, nucleic acid molecules encoding a variant Zcytor18 polypeptide remain hybridized with a nucleic acid molecule comprising the nucleotide sequence of nucleotides 192 to 652 of SEQ ID NO:1 (or its complement) under stringent washing conditions, in which the wash stringency is equivalent to 0.5x - 2x SSC with 0.1% SDS at 55 - 65°C, including 0.5x SSC with 0.1% SDS at 55°C, or 2xSSC with 0.1% SDS at 65°C. One of skill in the art can readily devise equivalent conditions, for example, by substituting SSPE for SSC in the wash solution.

Typical highly stringent washing conditions include washing in a solution of 0.1x - 0.2x SSC with 0.1% sodium dodecyl sulfate (SDS) at 50 - 65°C. For example, nucleic acid molecules encoding a variant Zcytor18 polypeptide remain hybridized with a nucleic acid molecule comprising the nucleotide sequence of nucleotides 192 to 652 of SEQ ID NO:1 (or its complement) under highly stringent washing conditions, in which the wash stringency is equivalent to 0.1x - 0.2x SSC with 0.1% SDS at 50 - 65°C, including 0.1x SSC with 0.1% SDS at 50°C, or 0.2xSSC with 0.1% SDS at 65°C.

The present invention also provides isolated Zcytor18 polypeptides that have a substantially similar sequence identity to the polypeptides of SEQ ID NO:2, SEQ ID NO:8, or their orthologs. The term "substantially similar sequence identity" is used herein to denote polypeptides having at least 70%, at least 80%, at least 90%, at least 95% or greater than 95% sequence identity to the sequences shown in SEQ ID NO:2, SEQ ID NO:8, or their orthologs.

The present invention also contemplates *Zcytor18* variant nucleic acid molecules that can be identified using two criteria: a determination of the similarity between the encoded polypeptide with the amino acid sequence of SEQ ID NO:2, and a hybridization assay, as described above. Such *Zcytor18* variants include nucleic acid molecules (1) that remain hybridized with a nucleic acid molecule comprising the nucleotide sequence of nucleotides 192 to 652 of SEQ ID NO:1 (or its complement) under stringent washing conditions, in which the wash stringency is equivalent to 0.5x - 2x SSC with 0.1% SDS at 55 - 65°C, and (2) that encode a polypeptide having at least 70%, at least 80%, at least 90%, at least 95% or greater than 95% sequence identity to the amino acid sequence of SEQ ID NO:2. Alternatively, *Zcytor18* variants can be characterized as nucleic acid molecules (1) that remain hybridized with a nucleic acid molecule comprising the nucleotide sequence of nucleotides 192 to 652 of SEQ ID NO:1 (or its complement) under highly stringent washing conditions, in which the wash stringency is equivalent to 0.1x - 0.2x SSC with 0.1% SDS at 50 - 65°C, and (2) that encode a polypeptide having at least 70%, at least 80%, at least 90%, at least 95% or greater than 95% sequence identity to the amino acid sequence of SEQ ID NO:2.

Percent sequence identity is determined by conventional methods. See, for example, Altschul *et al., Bull. Math. Bio. 48*:603 (1986), and Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA 89*:10915 (1992). Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "BLOSUM62" scoring matrix of Henikoff and Henikoff (*ibid.*) as shown in Table 3 (amino acids are indicated by the standard one-letter codes). The percent identity is then calculated as: ([Total number of identical matches]/ [length

of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences])(100).

Table 3

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

Those skilled in the art appreciate that there are many established algorithms available to align two amino acid sequences. The "FASTA" similarity search algorithm of Pearson and Lipman is a suitable protein alignment method for examining the level of identity shared by an amino acid sequence disclosed herein and the amino acid sequence of a putative Zcytor18 variant. The FASTA algorithm is described by Pearson and Lipman, *Proc. Nat'l Acad. Sci. USA 85*:2444 (1988), and by Pearson, *Meth. Enzymol. 183*:63 (1990). Briefly, FASTA first characterizes sequence similarity by identifying regions shared by the query sequence (*e.g.*, SEQ ID NO:2) and a test sequence that have either the highest density of identities (if the ktup variable is 1) or pairs of identities (if ktup=2), without considering conservative amino acid substitutions, insertions, or deletions. The ten regions with the highest density of identities are then rescored by comparing the similarity of all paired amino acids using an amino acid substitution matrix, and the ends of the regions are "trimmed" to include only those residues that contribute to the highest score. If there are several regions with scores greater than the "cutoff" value (calculated by a predetermined formula based upon the length of the sequence and the ktup value), then the trimmed initial regions are examined to determine whether the regions can be joined to form an approximate alignment with gaps. Finally, the highest scoring regions of the two amino acid sequences are aligned using a modification of the Needleman-Wunsch-Sellers algorithm (Needleman and Wunsch, *J. Mol. Biol. 48*:444 (1970); Sellers, *SIAM J. Appl. Math. 26*:787 (1974)), which allows for amino acid insertions and deletions. Illustrative parameters for FASTA analysis are: ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62. These parameters can be introduced into a FASTA program by modifying the scoring matrix file ("SMATRIX"), as explained in Appendix 2 of Pearson, *Meth. Enzymol. 183*:63 (1990).

FASTA can also be used to determine the sequence identity of nucleic acid molecules using a ratio as disclosed above. For nucleotide sequence comparisons, the ktup value can range between one to six, preferably from three to six, most preferably three, with other parameters set as described above.

The present invention includes nucleic acid molecules that encode a polypeptide having a conservative amino acid change, compared with an amino acid sequence disclosed herein. For example, variants can be obtained that contain one or more amino acid substitutions of SEQ ID NO:2, or SEQ ID NO:8, in which an alkyl amino acid is substituted for an alkyl amino acid in a Zcytor18 amino acid sequence, an aromatic amino acid is substituted for an aromatic amino acid in a Zcytor18 amino acid sequence, a sulfur-containing amino acid is substituted for a sulfur-containing amino acid in a Zcytor18 amino acid sequence, a hydroxy-containing amino acid is substituted

for a hydroxy-containing amino acid in a Zcytor18 amino acid sequence, an acidic amino acid is substituted for an acidic amino acid in a Zcytor18 amino acid sequence, a basic amino acid is substituted for a basic amino acid in a Zcytor18 amino acid sequence, or a dibasic monocarboxylic amino acid is substituted for a dibasic monocarboxylic amino acid in a Zcytor18 amino acid sequence. Among the common amino acids, for example, a "conservative amino acid substitution" is illustrated by a substitution among amino acids within each of the following groups: (1) glycine, alanine, valine, leucine, and isoleucine, (2) phenylalanine, tyrosine, and tryptophan, (3) serine and threonine, (4) aspartate and glutamate, (5) glutamine and asparagine, and (6) lysine, arginine and histidine.

The BLOSUM62 table is an amino acid substitution matrix derived from about 2,000 local multiple alignments of protein sequence segments, representing highly conserved regions of more than 500 groups of related proteins (Henikoff and Henikoff, *Proc. Nat'l Acad. Sci. USA 89*:10915 (1992)). Accordingly, the BLOSUM62 substitution frequencies can be used to define conservative amino acid substitutions that may be introduced into the amino acid sequences of the present invention. Although it is possible to design amino acid substitutions based solely upon chemical properties (as discussed above), the language "conservative amino acid substitution" preferably refers to a substitution represented by a BLOSUM62 value of greater than -1. For example, an amino acid substitution is conservative if the substitution is characterized by a BLOSUM62 value of 0, 1, 2, or 3. According to this system, preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 1 (*e.g.*, 1, 2 or 3), while more preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 2 (*e.g.*, 2 or 3).

Particular variants of Zcytor18 are characterized by having at least 70%, at least 80%, at least 90%, at least 95% or greater than 95% sequence identity to the corresponding amino acid sequence (*e.g.*, SEQ ID NO:2 or SEQ ID NO:8), wherein the variation in amino acid sequence is due to one or more conservative amino acid substitutions.

Conservative amino acid changes in a *Zcytor18* gene can be introduced, for example, by substituting nucleotides for the nucleotides recited in SEQ ID NO:1. Such "conservative amino acid" variants can be obtained by oligonucleotide-directed mutagenesis, linker-scanning mutagenesis, mutagenesis using the polymerase chain reaction, and the like (see Ausubel (1995) at pages 8-10 to 8-22; and McPherson (ed.), *Directed Mutagenesis: A Practical Approach* (IRL Press 1991)). A variant Zcytor18 polypeptide can be identified by the ability to specifically bind anti-Zcytor18 antibodies.

The proteins of the present invention can also comprise non-naturally occurring amino acid residues. Non-naturally occurring amino acids include, without limitation, *trans*-3-methylproline, 2,4-methanoproline, *cis*-4-hydroxyproline, *trans*-4-hydroxyproline, *N*-methylglycine, *allo*-threonine, methylthreonine, hydroxyethylcysteine, hydroxyethylhomocysteine, nitroglutamine, homoglutamine, pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, 3,3-dimethylproline, *tert*-leucine, norvaline, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-fluorophenylalanine. Several methods are known in the art for incorporating non-naturally occurring amino acid residues into proteins. For example, an *in vitro* system can be employed wherein nonsense mutations are suppressed using chemically aminoacylated suppressor tRNAs. Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations is typically carried out in a cell-free system comprising an *E. coli* S30 extract and commercially available enzymes and other reagents. Proteins are purified by chromatography. See, for example, Robertson *et al., J. Am. Chem. Soc. 113*:2722 (1991), Ellman *et al., Methods Enzymol. 202*:301 (1991), Chung *et al., Science 259*:806 (1993), and Chung *et al., Proc. Nat'l Acad. Sci. USA 90*:10145 (1993).

In a second method, translation is carried out in *Xenopus* oocytes by microinjection of mutated mRNA and chemically aminoacylated suppressor tRNAs (Turcatti *et al., J. Biol. Chem. 271*:19991 (1996)). Within a third method, *E. coli* cells are cultured in the absence of a natural amino acid that is to be replaced (*e.g.*, phenylalanine) and in the presence of the desired non-naturally occurring amino acid(s) (*e.g.*, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the protein in place of its natural counterpart. See, Koide *et al., Biochem. 33*:7470 (1994). Naturally occurring amino acid residues can be converted to non-naturally occurring species by *in vitro* chemical modification. Chemical modification can be combined with site-directed mutagenesis to further expand the range of substitutions (Wynn and Richards, *Protein Sci. 2*:395 (1993)).

A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, non-naturally occurring amino acids, and unnatural amino acids may be substituted for Zcytor18 amino acid residues.

Essential amino acids in the polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science 244*:1081 (1989), Bass *et al., Proc. Nat'l Acad. Sci. USA 88*:4498 (1991), Coombs and Corey, "Site-Directed Mutagenesis and Protein Engineering," in *Proteins: Analysis and Design*, Angeletti (ed.),

pages 259-311 (Academic Press, Inc. 1998)). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton *et al., J. Biol. Chem. 271*:4699 (1996).

Although sequence analysis can be used to further define the Zcytor18 ligand binding region, amino acids that play a role in Zcytor18 binding activity can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos *et al., Science 255*:306 (1992), Smith *et al., J. Mol. Biol. 224*:899 (1992), and Wlodaver *et al., FEBS Lett. 309*:59 (1992).

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer (*Science 241*:53 (1988)) or Bowie and Sauer (*Proc. Nat'l Acad. Sci. USA 86:*2152 (1989)). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display (*e.g.*, Lowman *et al., Biochem. 30*:10832 (1991), Ladner *et al.*, U.S. Patent No. 5,223,409, Huse, international publication No. WO 92/06204, and region-directed mutagenesis (Derbyshire *et al., Gene 46*:145 (1986), and Ner *et al., DNA 7*:127, (1988)). Moreover, Zcytor18 labeled with biotin or FITC can be used for expression cloning of Zcytor18 ligands.

Variants of the disclosed Zcytor18 nucleotide and polypeptide sequences can also be generated through DNA shuffling as disclosed by Stemmer, *Nature 370*:389 (1994), Stemmer, *Proc. Nat'l Acad. Sci. USA 91*:10747 (1994), and international publication No. WO 97/20078. Briefly, variant DNA molecules are generated by *in vitro* homologous recombination by random fragmentation of a parent DNA followed by reassembly using PCR, resulting in randomly introduced point mutations. This technique can be modified by using a family of parent DNA molecules, such as allelic variants or DNA molecules from different species, to introduce additional variability into the process. Selection or screening for the desired activity, followed by additional iterations of mutagenesis and assay provides for rapid "evolution" of sequences by selecting for desirable mutations while simultaneously selecting against detrimental changes.

Mutagenesis methods as disclosed herein can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides in host cells. Mutagenized DNA molecules that encode biologically active polypeptides, or polypeptides that bind with anti-Zcytor18 antibodies, can be recovered from the host cells and rapidly sequenced using modern equipment. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

The present invention also includes "functional fragments" of Zcytor18 polypeptides and nucleic acid molecules encoding such functional fragments. Routine deletion analyses of nucleic acid molecules can be performed to obtain functional fragments of a nucleic acid molecule that encodes a Zcytor18 polypeptide. As an illustration, DNA molecules comprising the nucleotide sequence of nucleotides 192 to 2347 of SEQ ID NO:1 can be digested with *Bal*31 nuclease to obtain a series of nested deletions. The fragments are then inserted into expression vectors in proper reading frame, and the expressed polypeptides are isolated and tested for the ability to bind anti-Zcytor18 antibodies. One alternative to exonuclease digestion is to use oligonucleotide-directed mutagenesis to introduce deletions or stop codons to specify production of a desired fragment. Alternatively, particular fragments of a *Zcytor18* gene can be synthesized using the polymerase chain reaction. An example of a functional fragment is the extracellular domain of Zcytor18 (*i.e.*, amino acid residues 36 to 313 of SEQ ID NO:2 or SEQ ID NO:5, or amino acid residues 36 to 299 of SEQ ID NO:8).

This general approach is exemplified by studies on the truncation at either or both termini of interferons have been summarized by Horisberger and Di Marco, *Pharmac. Ther. 66*:507 (1995). Moreover, standard techniques for functional analysis of proteins are described by, for example, Treuter *et al.*, *Molec. Gen. Genet. 240*:113 (1993), Content *et al.*, "Expression and preliminary deletion analysis of the 42 kDa 2-5A synthetase induced by human interferon," in *Biological Interferon Systems, Proceedings of ISIR-TNO Meeting on Interferon Systems*, Cantell (ed.), pages 65-72 (Nijhoff 1987), Herschman, "The EGF Receptor," in *Control of Animal Cell Proliferation, Vol. 1*, Boynton *et al.*, (eds.) pages 169-199 (Academic Press 1985), Coumailleau *et al., J. Biol. Chem. 270*:29270 (1995); Fukunaga *et al., J. Biol. Chem. 270*:25291 (1995); Yamaguchi *et al., Biochem. Pharmacol. 50*:1295 (1995), and Meisel *et al., Plant Molec. Biol. 30*:1 (1996).

The present invention also contemplates functional fragments of a *Zcytor18* gene that have amino acid changes, compared with an amino acid sequence disclosed herein. A variant *Zcytor18* gene can be identified on the basis of structure by determining the level of identity with disclosed nucleotide and amino acid sequences, as

discussed above. An alternative approach to identifying a variant gene on the basis of structure is to determine whether a nucleic acid molecule encoding a potential variant *Zcytor18* gene can hybridize to a nucleic acid molecule comprising a nucleotide sequence, such as SEQ ID NO:1.

The present invention also provides polypeptide fragments or peptides comprising an epitope-bearing portion of a Zcytor18 polypeptide described herein. Such fragments or peptides may comprise an "immunogenic epitope," which is a part of a protein that elicits an antibody response when the entire protein is used as an immunogen. Immunogenic epitope-bearing peptides can be identified using standard methods (see, for example, Geysen *et al., Proc. Nat'l Acad. Sci. USA 81*:3998 (1983)).

In contrast, polypeptide fragments or peptides may comprise an "antigenic epitope," which is a region of a protein molecule to which an antibody can specifically bind. Certain epitopes consist of a linear or contiguous stretch of amino acids, and the antigenicity of such an epitope is not disrupted by denaturing agents. It is known in the art that relatively short synthetic peptides that can mimic epitopes of a protein can be used to stimulate the production of antibodies against the protein (see, for example, Sutcliffe *et al., Science 219*:660 (1983)).)). Antibodies that recognize short linear epitopes are particularly useful in analytic and diagnostic applications that use denatured protein, such as Western analysis, or in the analysis of fixed cells or tissue samples. Antibodies to linear epitopes are also useful for detecting fragments of Zcytor18, such as might occur in body fluids or culture media. Accordingly, antigenic epitope-bearing peptides and polypeptides of the present invention are useful to raise antibodies that bind with the polypeptides described herein.

Antigenic epitope-bearing peptides and polypeptides can contain at least four to ten amino acids, at least ten to fifteen amino acids, or about 15 to about 30 amino acids of an amino acid sequence disclosed herein. Such epitope-bearing peptides and polypeptides can be produced by fragmenting a Zcytor18 polypeptide, or by chemical peptide synthesis, as described herein. Moreover, epitopes can be selected by phage display of random peptide libraries (see, for example, Lane and Stephen, *Curr. Opin. Immunol. 5*:268 (1993), and Cortese *et al., Curr. Opin. Biotechnol. 7*:616 (1996)). Standard methods for identifying epitopes and producing antibodies from small peptides that comprise an epitope are described, for example, by Mole, "Epitope Mapping," in *Methods in Molecular Biology, Vol. 10*, Manson (ed.), pages 105-116 (The Humana Press, Inc. 1992), Price, "Production and Characterization of Synthetic Peptide-Derived Antibodies," in *Monoclonal Antibodies: Production, Engineering, and Clinical Application*, Ritter and Ladyman (eds.), pages 60-84 (Cambridge University Press 1995),

and Coligan *et al.* (eds.), *Current Protocols in Immunology*, pages 9.3.1 - 9.3.5 and pages 9.4.1 - 9.4.11 (John Wiley & Sons 1997).

For any Zcytor18 polypeptide, including variants and fusion proteins, one of ordinary skill in the art can readily generate a fully degenerate polynucleotide sequence encoding that variant using the information set forth in Tables 1 and 2 above. Moreover, those of skill in the art can use standard software to devise Zcytor18 variants based upon the nucleotide and amino acid sequences described herein. Accordingly, the present invention includes a computer-readable medium encoded with a data structure that provides at least one of the following sequences: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9. Suitable forms of computer-readable media include magnetic media and optically-readable media. Examples of magnetic media include a hard or fixed drive, a random access memory (RAM) chip, a floppy disk, digital linear tape (DLT), a disk cache, and a ZIP disk. Optically readable media are exemplified by compact discs (*e.g.*, CD-read only memory (ROM), CD-rewritable (RW), and CD-recordable), and digital versatile/video discs (DVD) (*e.g.*, DVD-ROM, DVD-RAM, and DVD+RW).

5. Production of Zcytor18 Polypeptides

The polypeptides of the present invention, including full-length polypeptides, functional fragments, and fusion proteins, can be produced in recombinant host cells following conventional techniques. To express a *Zcytor18* gene, a nucleic acid molecule encoding the polypeptide must be operably linked to regulatory sequences that control transcriptional expression in an expression vector and then, introduced into a host cell. In addition to transcriptional regulatory sequences, such as promoters and enhancers, expression vectors can include translational regulatory sequences and a marker gene which is suitable for selection of cells that carry the expression vector.

Expression vectors that are suitable for production of a foreign protein in eukaryotic cells typically contain (1) prokaryotic DNA elements coding for a bacterial replication origin and an antibiotic resistance marker to provide for the growth and selection of the expression vector in a bacterial host; (2) eukaryotic DNA elements that control initiation of transcription, such as a promoter; and (3) DNA elements that control the processing of transcripts, such as a transcription termination/polyadenylation sequence. As discussed above, expression vectors can also include nucleotide sequences encoding a secretory sequence that directs the heterologous polypeptide into the secretory pathway of a host cell. For example, a *Zcytor18* expression vector may comprise a *Zcytor18* gene and a secretory sequence derived from any secreted gene.

Zcytor18 proteins of the present invention may be expressed in mammalian cells. Examples of suitable mammalian host cells include African green monkey kidney cells (Vero; ATCC CRL 1587), human embryonic kidney cells (293-HEK; ATCC CRL 1573), baby hamster kidney cells (BHK-21, BHK-570; ATCC CRL 8544, ATCC CRL 10314), canine kidney cells (MDCK; ATCC CCL 34), Chinese hamster ovary cells (CHO-K1; ATCC CCL61; CHO DG44 (Chasin *et al., Som. Cell. Molec. Genet. 12*:555, 1986)), rat pituitary cells (GH1; ATCC CCL82), HeLa S3 cells (ATCC CCL2.2), rat hepatoma cells (H-4-II-E; ATCC CRL 1548) SV40-transformed monkey kidney cells (COS-1; ATCC CRL 1650) and murine embryonic cells (NIH-3T3; ATCC CRL 1658).

For a mammalian host, the transcriptional and translational regulatory signals may be derived from viral sources, such as adenovirus, bovine papilloma virus, simian virus, or the like, in which the regulatory signals are associated with a particular gene which has a high level of expression. Suitable transcriptional and translational regulatory sequences also can be obtained from mammalian genes, such as actin, collagen, myosin, and metallothionein genes.

Transcriptional regulatory sequences include a promoter region sufficient to direct the initiation of RNA synthesis. Suitable eukaryotic promoters include the promoter of the mouse *metallothionein I* gene (Hamer *et al., J. Molec. Appl. Genet. 1*:273 (1982)), the *TK* promoter of *Herpes* virus (McKnight, *Cell 31*:355 (1982)), the *SV40* early promoter (Benoist *et al., Nature 290*:304 (1981)), the *Rous* sarcoma virus promoter (Gorman *et al., Proc. Nat'l Acad. Sci. USA 79*:6777 (1982)), the cytomegalovirus promoter (Foecking *et al., Gene 45*:101 (1980)), and the mouse mammary tumor virus promoter (see, generally, Etcheverry, "Expression of Engineered Proteins in Mammalian Cell Culture," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 163-181 (John Wiley & Sons, Inc. 1996)).

Alternatively, a prokaryotic promoter, such as the bacteriophage T3 RNA polymerase promoter, can be used to control *Zcytor18* gene expression in mammalian cells if the prokaryotic promoter is regulated by a eukaryotic promoter (Zhou *et al., Mol. Cell. Biol. 10*:4529 (1990), and Kaufman *et al., Nucl. Acids Res. 19*:4485 (1991)).

An expression vector can be introduced into host cells using a variety of standard techniques including calcium phosphate transfection, liposome-mediated transfection, microprojectile-mediated delivery, electroporation, and the like. The transfected cells can be selected and propagated to provide recombinant host cells that comprise the expression vector stably integrated in the host cell genome. Techniques for introducing vectors into eukaryotic cells and techniques for selecting such stable transformants using a dominant selectable marker are described, for example, by Ausubel

(1995) and by Murray (ed.), *Gene Transfer and Expression Protocols* (Humana Press 1991).

For example, one suitable selectable marker is a gene that provides resistance to the antibiotic neomycin. In this case, selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems can also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. A suitable amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (*e.g.*, hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used. Alternatively, markers that introduce an altered phenotype, such as green fluorescent protein, or cell surface proteins such as CD4, CD8, Class I MHC, placental alkaline phosphatase may be used to sort transfected cells from untransfected cells by such means as FACS sorting or magnetic bead separation technology.

Zcytor18 polypeptides can also be produced by cultured mammalian cells using a viral delivery system. Exemplary viruses for this purpose include adenovirus, herpesvirus, vaccinia virus and adeno-associated virus (AAV). Adenovirus, a double-stranded DNA virus, is currently the best studied gene transfer vector for delivery of heterologous nucleic acid (for a review, see Becker *et al., Meth. Cell Biol. 43*:161 (1994), and Douglas and Curiel, *Science & Medicine 4*:44 (1997)). Advantages of the adenovirus system include the accommodation of relatively large DNA inserts, the ability to grow to high-titer, the ability to infect a broad range of mammalian cell types, and flexibility that allows use with a large number of available vectors containing different promoters.

By deleting portions of the adenovirus genome, larger inserts (up to 7 kb) of heterologous DNA can be accommodated. These inserts can be incorporated into the viral DNA by direct ligation or by homologous recombination with a co-transfected plasmid. An option is to delete the essential *E1* gene from the viral vector, which results in the inability to replicate unless the *E1* gene is provided by the host cell. Adenovirus vector-infected human 293 cells (ATCC Nos. CRL-1573, 45504, 45505), for example, can be grown as adherent cells or in suspension culture at relatively high cell density to produce significant amounts of protein (see Garnier *et al., Cytotechnol. 15*:145 (1994)).

Zcytor18 can also be expressed in other higher eukaryotic cells, such as avian, fungal, insect, yeast, or plant cells. The baculovirus system provides an efficient means to introduce cloned *Zcytor18* genes into insect cells. Suitable expression vectors

are based upon the *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV), and contain well-known promoters such as *Drosophila heat shock protein* (*hsp*) 70 promoter, *Autographa californica nuclear polyhedrosis virus immediate-early* gene promoter (*ie-1*) and the *delayed early 39K* promoter, baculovirus *p10* promoter, and the *Drosophila metallothionein* promoter. A second method of making recombinant baculovirus utilizes a transposon-based system described by Luckow (Luckow, *et al., J. Virol. 67*:4566 (1993)). This system, which utilizes transfer vectors, is sold in the BAC-to-BAC kit (Life Technologies, Rockville, MD). This system utilizes a transfer vector, PFASTBAC (Life Technologies) containing a Tn7 transposon to move the DNA encoding the Zcytor18 polypeptide into a baculovirus genome maintained in *E. coli* as a large plasmid called a "bacmid." See, Hill-Perkins and Possee, *J. Gen. Virol. 71*:971 (1990), Bonning, *et al., J. Gen. Virol. 75*:1551 (1994), and Chazenbalk, and Rapoport, *J. Biol. Chem. 270*:1543 (1995). In addition, transfer vectors can include an in-frame fusion with DNA encoding an epitope tag at the C- or N-terminus of the expressed Zcytor18 polypeptide, for example, a Glu-Glu epitope tag (Grussenmeyer *et al., Proc. Nat'l Acad. Sci. 82*:7952 (1985)). Using a technique known in the art, a transfer vector containing a *Zcytor18* gene is transformed into *E. coli*, and screened for bacmids, which contain an interrupted *lacZ* gene indicative of recombinant baculovirus. The bacmid DNA containing the recombinant baculovirus genome is then isolated using common techniques.

The illustrative PFASTBAC vector can be modified to a considerable degree. For example, the polyhedrin promoter can be removed and substituted with the baculovirus basic protein promoter (also known as P*cor*, p6.9 or MP promoter) which is expressed earlier in the baculovirus infection, and has been shown to be advantageous for expressing secreted proteins (see, for example, Hill-Perkins and Possee, *J. Gen. Virol. 71*:971 (1990), Bonning, *et al., J. Gen. Virol. 75*:1551 (1994), and Chazenbalk and Rapoport, *J. Biol. Chem. 270*:1543 (1995). In such transfer vector constructs, a short or long version of the basic protein promoter can be used. Moreover, transfer vectors can be constructed, which replace the native Zcytor18 secretory signal sequences with secretory signal sequences derived from insect proteins. For example, a secretory signal sequence from Ecdysteroid Glucosyltransferase (EGT), honey bee Melittin (Invitrogen Corporation; Carlsbad, CA), or baculovirus gp67 (PharMingen: San Diego, CA) can be used in constructs to replace the native Zcytor18 secretory signal sequence.

The recombinant virus or bacmid is used to transfect host cells. Suitable insect host cells include cell lines derived from IPLB-*Sf*-21, a *Spodoptera frugiperda* pupal ovarian cell line, such as *Sf*9 (ATCC CRL 1711), *Sf*21AE, and *Sf*21 (Invitrogen Corporation; San Diego, CA), as well as *Drosophila* Schneider-2 cells, and the HIGH

FIVEO cell line (Invitrogen) derived from *Trichoplusia ni* (U.S. Patent No. 5,300,435). Commercially available serum-free media can be used to grow and to maintain the cells. Suitable media are Sf900 II™ (Life Technologies) or ESF 921™ (Expression Systems) for the Sf9 cells; and Ex-cellO405™ (JRH Biosciences, Lenexa, KS) or Express FiveO™ (Life Technologies) for the *T. ni* cells. When recombinant virus is used, the cells are typically grown up from an inoculation density of approximately 2-5 x 10^5 cells to a density of 1-2 x 10^6 cells at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of 0.1 to 10, more typically near 3.

Established techniques for producing recombinant proteins in baculovirus systems are provided by Bailey *et al.*, "Manipulation of Baculovirus Vectors," in *Methods in Molecular Biology, Volume 7: Gene Transfer and Expression Protocols*, Murray (ed.), pages 147-168 (The Humana Press, Inc. 1991), by Patel *et al.*, "The baculovirus expression system," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), pages 205-244 (Oxford University Press 1995), by Ausubel (1995) at pages 16-37 to 16-57, by Richardson (ed.), *Baculovirus Expression Protocols* (The Humana Press, Inc. 1995), and by Lucknow, "Insect Cell Expression Technology," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 183-218 (John Wiley & Sons, Inc. 1996).

Fungal cells, including yeast cells, can also be used to express the genes described herein. Yeast species of particular interest in this regard include *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Pichia methanolica*. Suitable promoters for expression in yeast include promoters from *GAL1* (galactose), *PGK* (phosphoglycerate kinase), *ADH* (alcohol dehydrogenase), *AOX1* (alcohol oxidase), HIS4 (histidinol dehydrogenase), and the like. Many yeast cloning vectors have been designed and are readily available. These vectors include YIp-based vectors, such as YIp5, YRp vectors, such as YRp17, YEp vectors such as YEp13 and YCp vectors, such as YCp19. Methods for transforming *S. cerevisiae* cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311, Kawasaki *et al.*, U.S. Patent No. 4,931,373, Brake, U.S. Patent No. 4,870,008, Welch *et al.*, U.S. Patent No. 5,037,743, and Murray *et al.*, U.S. Patent No. 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (*e.g.*, leucine). A suitable vector system for use in *Saccharomyces cerevisiae* is the *POT1* vector system disclosed by Kawasaki *et al.* (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Additional suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, *e.g.*, Kawasaki, U.S. Patent No. 4,599,311,

Kingsman *et al.*, U.S. Patent No. 4,615,974, and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446, 5,063,154, 5,139,936, and 4,661,454.

Transformation systems for other yeasts, including *Hansenula polymorpha, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces fragilis, Ustilago maydis, Pichia pastoris, Pichia methanolica, Pichia guillermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson *et al., J. Gen. Microbiol. 132*:3459 (1986), and Cregg, U.S. Patent No. 4,882,279. *Aspergillus* cells may be utilized according to the methods of McKnight *et al.*, U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino *et al.*, U.S. Patent No. 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533.

For example, the use of *Pichia methanolica* as host for the production of recombinant proteins is disclosed by Raymond, U.S. Patent No. 5,716,808, Raymond, U.S. Patent No. 5,736,383, Raymond *et al., Yeast 14*:11-23 (1998), and in international publication Nos. WO 97/17450, WO 97/17451, WO 98/02536, and WO 98/02565. DNA molecules for use in transforming *P. methanolica* will commonly be prepared as double-stranded, circular plasmids, which can be linearized prior to transformation. For polypeptide production in *P. methanolica*, the promoter and terminator in the plasmid can be that of a *P. methanolica* gene, such as a *P. methanolica* alcohol utilization gene (*AUG1* or *AUG2*). Other useful promoters include those of the dihydroxyacetone synthase (DHAS), formate dehydrogenase (FMD), and catalase (CAT) genes. To facilitate integration of the DNA into the host chromosome, the entire expression segment of the plasmid can be flanked at both ends by host DNA sequences. A suitable selectable marker for use in *Pichia methanolica* is a *P. methanolica ADE2* gene, which encodes phosphoribosyl-5-aminoimidazole carboxylase (AIRC; EC 4.1.1.21), and which allows *ade2* host cells to grow in the absence of adenine. For large-scale, industrial processes where it is desirable to minimize the use of methanol, host cells can be used in which both methanol utilization genes (*AUG1* and *AUG2*) are deleted. For production of secreted proteins, host cells can be deficient in vacuolar protease genes (*PEP4* and *PRB1*). Electroporation is used to facilitate the introduction of a plasmid containing DNA encoding a polypeptide of interest into *P. methanolica* cells. *P. methanolica* cells can be transformed by electroporation using an exponentially decaying, pulsed electric field having a field strength of from 2.5 to 4.5 kV/cm, preferably about 3.75 kV/cm, and a time constant (t) of from 1 to 40 milliseconds, most preferably about 20 milliseconds.

Expression vectors can also be introduced into plant protoplasts, intact plant tissues, or isolated plant cells. Methods for introducing expression vectors into plant tissue

include the direct infection or co-cultivation of plant tissue with *Agrobacterium tumefaciens*, microprojectile-mediated delivery, DNA injection, electroporation, and the like. See, for example, Horsch *et al., Science* 227:1229 (1985), Klein *et al., Biotechnology* 10:268 (1992), and Miki *et al.*, "Procedures for Introducing Foreign DNA into Plants," in *Methods in Plant Molecular Biology and Biotechnology*, Glick *et al.* (eds.), pages 67-88 (CRC Press, 1993).

Alternatively, *Zcytor18* genes can be expressed in prokaryotic host cells. Suitable promoters that can be used to express Zcytor18 polypeptides in a prokaryotic host are well-known to those of skill in the art and include promoters capable of recognizing the T4, T3, Sp6 and T7 polymerases, the P_R and P_L promoters of bacteriophage lambda, the *trp*, *recA*, heat shock, *lacUV5*, *tac*, *lpp-lacSpr*, *phoA*, and *lacZ* promoters of *E. coli*, promoters of *B. subtilis*, the promoters of the bacteriophages of *Bacillus*, *Streptomyces* promoters, the *int* promoter of bacteriophage lambda, the *bla* promoter of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene. Prokaryotic promoters have been reviewed by Glick, *J. Ind. Microbiol. 1*:277 (1987), Watson *et al., Molecular Biology of the Gene, 4th Ed.* (Benjamin Cummins 1987), and by Ausubel *et al.* (1995).

Suitable prokaryotic hosts include *E. coli* and *Bacillus subtilus*. Suitable strains of *E. coli* include BL21(DE3), BL21(DE3)pLysS, BL21(DE3)pLysE, DH1, DH4I, DH5, DH5I, DH5IF', DH5IMCR, DH10B, DH10B/p3, DH11S, C600, HB101, JM101, JM105, JM109, JM110, K38, RR1, Y1088, Y1089, CSH18, ER1451, and ER1647 (see, for example, Brown (ed.), *Molecular Biology Labfax* (Academic Press 1991)). Suitable strains of *Bacillus subtilus* include BR151, YB886, MI119, MI120, and B170 (see, for example, Hardy, "Bacillus Cloning Methods," in *DNA Cloning: A Practical Approach*, Glover (ed.) (IRL Press 1985)).

When expressing a Zcytor18 polypeptide in bacteria such as *E. coli*, the polypeptide may be retained in the cytoplasm, typically as insoluble granules, or may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example, guanidine isothiocyanate or urea. The denatured polypeptide can then be refolded and dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of reduced and oxidized glutathione, followed by dialysis against a buffered saline solution. In the latter case, the polypeptide can be recovered from the periplasmic space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) to release the contents of the periplasmic space and recovering the protein, thereby obviating the need for denaturation and refolding.

Methods for expressing proteins in prokaryotic hosts are well-known to those of skill in the art (see, for example, Williams *et al.*, "Expression of foreign proteins in *E. coli* using plasmid vectors and purification of specific polyclonal antibodies," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), page 15 (Oxford University Press 1995), Ward *et al.*, "Genetic Manipulation and Expression of Antibodies," in *Monoclonal Antibodies: Principles and Applications*, page 137 (Wiley-Liss, Inc. 1995), and Georgiou, "Expression of Proteins in Bacteria," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), page 101 (John Wiley & Sons, Inc. 1996)).

Standard methods for introducing expression vectors into bacterial, yeast, insect, and plant cells are provided, for example, by Ausubel (1995).

General methods for expressing and recovering foreign protein produced by a mammalian cell system are provided by, for example, Etcheverry, "Expression of Engineered Proteins in Mammalian Cell Culture," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 163 (Wiley-Liss, Inc. 1996). Standard techniques for recovering protein produced by a bacterial system is provided by, for example, Grisshammer *et al.*, "Purification of over-produced proteins from *E. coli* cells," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), pages 59-92 (Oxford University Press 1995). Established methods for isolating recombinant proteins from a baculovirus system are described by Richardson (ed.), *Baculovirus Expression Protocols* (The Humana Press, Inc. 1995).

As an alternative, polypeptides of the present invention can be synthesized by exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. These synthesis methods are well-known to those of skill in the art (see, for example, Merrifield, *J. Am. Chem. Soc. 85*:2149 (1963), Stewart *et al.*, "Solid Phase Peptide Synthesis" (2nd Edition), (Pierce Chemical Co. 1984), Bayer and Rapp, *Chem. Pept. Prot. 3*:3 (1986), Atherton *et al., Solid Phase Peptide Synthesis: A Practical Approach* (IRL Press 1989), Fields and Colowick, "Solid-Phase Peptide Synthesis," *Methods in Enzymology Volume 289* (Academic Press 1997), and Lloyd-Williams *et al., Chemical Approaches to the Synthesis of Peptides and Proteins* (CRC Press, Inc. 1997)). Variations in total chemical synthesis strategies, such as "native chemical ligation" and "expressed protein ligation" are also standard (see, for example, Dawson *et al., Science 266*:776 (1994), Hackeng *et al., Proc. Nat'l Acad. Sci. USA 94*:7845 (1997), Dawson, *Methods Enzymol. 287*: 34 (1997), Muir *et al, Proc. Nat'l Acad. Sci. USA 95*:6705 (1998), and Severinov and Muir, *J. Biol. Chem. 273*:16205 (1998)).

Peptides and polypeptides of the present invention comprise at least six, at least nine, or at least 15 contiguous amino acid residues of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:8. As an illustration, polypeptides can comprise at least six, at least nine, or at least 15 contiguous amino acid residues of any of the following amino acid sequences: (a) amino acid residues 1 to 203 of SEQ ID NO:2, (b) amino acid residues 36 to 203 of SEQ ID NO:2, (c) amino acid residues 36 to 313 of SEQ ID NO:2, (d) amino acid residues 1 to 753 of SEQ ID NO:2, (e) amino acid residues 1 to 189 of SEQ ID NO:8, (f) amino acid residues 36 to 189 of SEQ ID NO:8, (g) amino acid residues 36 to 299 of SEQ ID NO:8, and (h) amino acid residues 1 to 739 of SEQ ID NO:8. Within certain embodiments of the invention, the polypeptides comprise 20, 30, 40, 50, 100, or more contiguous residues of these amino acid sequences. For example, polypeptides can comprise at least 30 contiguous amino acid residues of an amino acid sequence selected from the group consisting of: (a) amino acid residues 1 to 218 of SEQ ID NO:2, (b) amino acid residues 36 to 218 of SEQ ID NO:2, (c) amino acid residues 36 to 313 of SEQ ID NO:2, (d) amino acid residues 1 to 753 of SEQ ID NO:2, (e) amino acid residues 1 to 204 of SEQ ID NO:8, (f) amino acid residues 36 to 204 of SEQ ID NO:8, (g) amino acid residues 36 to 299 of SEQ ID NO:8, and (h) amino acid residues 1 to 739 of SEQ ID NO:8. Nucleic acid molecules encoding such peptides and polypeptides are useful as polymerase chain reaction primers and probes.

6. *Production of Zcytor18 Fusion Proteins and Conjugates*

One general class of Zcytor18 analogs are variants having an amino acid sequence that is a mutation of the amino acid sequence disclosed herein. Another general class of Zcytor18 analogs is provided by anti-idiotype antibodies, and fragments thereof, as described below. Moreover, recombinant antibodies comprising anti-idiotype variable domains can be used as analogs (see, for example, Monfardini *et al., Proc. Assoc. Am. Physicians 108*:420 (1996)). Since the variable domains of anti-idiotype Zcytor18 antibodies mimic Zcytor18, these domains can provide Zcytor18 binding activity. Methods of producing anti-idiotypic catalytic antibodies are known to those of skill in the art (see, for example, Joron *et al., Ann. N Y Acad. Sci. 672*:216 (1992), Friboulet *et al., Appl. Biochem. Biotechnol. 47*:229 (1994), and Avalle *et al., Ann. N Y Acad. Sci. 864*:118 (1998)).

Another approach to identifying Zcytor18 analogs is provided by the use of combinatorial libraries. Methods for constructing and screening phage display and other combinatorial libraries are provided, for example, by Kay *et al., Phage Display of*

Peptides and Proteins (Academic Press 1996), Verdine, U.S. Patent No. 5,783,384, Kay, *et. al.*, U.S. Patent No. 5,747,334, and Kauffman *et al.*, U.S. Patent No. 5,723,323.

Zcytor18 polypeptides have both *in vivo* and *in vitro* uses. As an illustration, a soluble form of Zcytor18 can be added to cell culture medium to inhibit the effects of the Zcytor18 ligand produced by the cultured cells.

Fusion proteins of Zcytor18 can be used to express *Zcytor18* in a recombinant host, and to isolate the produced Zcytor18. As described below, particular Zcytor18 fusion proteins also have uses in diagnosis and therapy. One type of fusion protein comprises a peptide that guides a Zcytor18 polypeptide from a recombinant host cell. To direct a Zcytor18 polypeptide into the secretory pathway of a eukaryotic host cell, a secretory signal sequence (also known as a signal peptide, a leader sequence, prepro sequence or pre sequence) is provided in the *Zcytor18* expression vector. While the secretory signal sequence may be derived from Zcytor18, a suitable signal sequence may also be derived from another secreted protein or synthesized *de novo*. The secretory signal sequence is operably linked to a *Zcytor18*-encoding sequence such that the two sequences are joined in the correct reading frame and positioned to direct the newly synthesized polypeptide into the secretory pathway of the host cell. Secretory signal sequences are commonly positioned 5' to the nucleotide sequence encoding the polypeptide of interest, although certain secretory signal sequences may be positioned elsewhere in the nucleotide sequence of interest (see, *e.g.*, Welch *et al.*, U.S. Patent No. 5,037,743; Holland *et al.*, U.S. Patent No. 5,143,830).

Although the secretory signal sequence of Zcytor18 or another protein produced by mammalian cells (*e.g.*, tissue-type plasminogen activator signal sequence, as described, for example, in U.S. Patent No. 5,641,655) is useful for expression of Zcytor18 in recombinant mammalian hosts, a yeast signal sequence is preferred for expression in yeast cells. Examples of suitable yeast signal sequences are those derived from yeast mating phermone α-factor (encoded by the *MFα1* gene), invertase (encoded by the *SUC2* gene), or acid phosphatase (encoded by the *PHO5* gene). See, for example, Romanos *et al.*, "Expression of Cloned Genes in Yeast," in *DNA Cloning 2: A Practical Approach*, 2nd Edition, Glover and Hames (eds.), pages 123-167 (Oxford University Press 1995).

In bacterial cells, it is often desirable to express a heterologous protein as a fusion protein to decrease toxicity, increase stability, and to enhance recovery of the expressed protein. For example, *Zcytor18* can be expressed as a fusion protein comprising a glutathione S-transferase polypeptide. Glutathione S-transferease fusion proteins are typically soluble, and easily purifiable from *E. coli* lysates on immobilized glutathione columns. In similar approaches, a Zcytor18 fusion protein comprising a

maltose binding protein polypeptide can be isolated with an amylose resin column, while a fusion protein comprising the C-terminal end of a truncated Protein A gene can be purified using IgG-Sepharose. Established techniques for expressing a heterologous polypeptide as a fusion protein in a bacterial cell are described, for example, by Williams *et al.*, "Expression of Foreign Proteins in *E. coli* Using Plasmid Vectors and Purification of Specific Polyclonal Antibodies," in *DNA Cloning 2: A Practical Approach,* 2nd Edition, Glover and Hames (Eds.), pages 15-58 (Oxford University Press 1995). In addition, commercially available expression systems are available. For example, the PINPOINT Xa protein purification system (Promega Corporation; Madison, WI) provides a method for isolating a fusion protein comprising a polypeptide that becomes biotinylated during expression with a resin that comprises avidin.

Peptide tags that are useful for isolating heterologous polypeptides expressed by either prokaryotic or eukaryotic cells include polyHistidine tags (which have an affinity for nickel-chelating resin), *c-myc* tags, calmodulin binding protein (isolated with calmodulin affinity chromatography), substance P, the RYIRS tag (which binds with anti-RYIRS antibodies), the Glu-Glu tag, and the FLAG tag (which binds with anti-FLAG antibodies). See, for example, Luo *et al., Arch. Biochem. Biophys. 329*:215 (1996), Morganti *et al., Biotechnol. Appl. Biochem. 23*:67 (1996), and Zheng *et al., Gene 186*:55 (1997). Nucleic acid molecules encoding such peptide tags are available, for example, from Sigma-Aldrich Corporation (St. Louis, MO).

The present invention also contemplates that the use of the secretory signal sequence contained in the Zcytor18 polypeptides of the present invention to direct other polypeptides into the secretory pathway. A signal fusion polypeptide can be made wherein a secretory signal sequence derived from amino acid residues 1 to 35 of SEQ ID NO:2 is operably linked to another polypeptide using methods known in the art and disclosed herein. The secretory signal sequence contained in the fusion polypeptides of the present invention is preferably fused amino-terminally to an additional peptide to direct the additional peptide into the secretory pathway. Such constructs have numerous applications known in the art. For example, these novel secretory signal sequence fusion constructs can direct the secretion of an active component of a normally non-secreted protein, such as a receptor. Such fusions may be used in a transgenic animal or in a cultured recombinant host to direct peptides through the secretory pathway. With regard to the latter, exemplary polypeptides include pharmaceutically active molecules such as Factor VIIa, proinsulin, insulin, follicle stimulating hormone, tissue type plasminogen activator, tumor necrosis factor, interleukins (*e.g.*, interleukin-1 (IL-1), IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21), colony stimulating factors (*e.g.*, granulocyte-colony stimulating

factor (G-CSF) and granulocyte macrophage-colony stimulating factor (GM-CSF)), interferons (*e.g.*, interferons-α, -β, -γ, -ω, -δ, -ε, and -τ), the stem cell growth factor designated "S1 factor," erythropoietin, and thrombopoietin. The Zcytor18 secretory signal sequence contained in the fusion polypeptides of the present invention is preferably fused amino-terminally to an additional peptide to direct the additional peptide into the secretory pathway. Fusion proteins comprising a Zcytor18 secretory signal sequence can be constructed using standard techniques.

Another form of fusion protein comprises a Zcytor18 polypeptide and an immunoglobulin heavy chain constant region, typically an F_c fragment, which contains two or three constant region domains and a hinge region but lacks the variable region. As an illustration, Chang *et al.*, U.S. Patent No. 5,723,125, describe a fusion protein comprising a human interferon and a human immunoglobulin Fc fragment. The C-terminal of the interferon is linked to the N-terminal of the Fc fragment by a peptide linker moiety. An example of a peptide linker is a peptide comprising primarily a T cell inert sequence, which is immunologically inert. An exemplary peptide linker has the amino acid sequence: GGSGG SGGGG SGGGG S (SEQ ID NO:10). In this fusion protein, an illustrative Fc moiety is a human γ4 chain, which is stable in solution and has little or no complement activating activity. Accordingly, the present invention contemplates a Zcytor18 fusion protein that comprises a Zcytor18 moiety and a human Fc fragment, wherein the C-terminus of the Zcytor18 moiety is attached to the N-terminus of the Fc fragment via a peptide linker, such as a peptide consisting of the amino acid sequence of SEQ ID NO:10. The Zcytor18 moiety can be a Zcytor18 molecule or a fragment thereof. For example, a fusion protein can comprise an Fc fragment (*e.g.*, a human Fc fragment), and amino acid residues 36 to 313 of SEQ ID NO:2, amino acid residues 36 to 189 of SEQ ID NO:2, amino acid residues 36 to 299 of SEQ ID NO:8, or amino acid residues 36 to 175 of SEQ ID NO:8.

In another variation, a Zcytor18 fusion protein comprises an IgG sequence, a Zcytor18 moiety covalently joined to the aminoterminal end of the IgG sequence, and a signal peptide that is covalently joined to the aminoterminal of the Zcytor18 moiety, wherein the IgG sequence consists of the following elements in the following order: a hinge region, a CH_2 domain, and a CH_3 domain. Accordingly, the IgG sequence lacks a CH_1 domain. The Zcytor18 moiety displays a Zcytor18 activity, as described herein, such as the ability to bind with a Zcytor18 ligand. This general approach to producing fusion proteins that comprise both antibody and nonantibody portions has been described by LaRochelle *et al.*, EP 742830 (WO 95/21258).

Fusion proteins comprising a Zcytor18 moiety and an Fc moiety can be used, for example, as an *in vitro* assay tool. For example, the presence of a Zcytor18

ligand in a biological sample can be detected using a Zcytor18-immunoglobulin fusion protein, in which the Zcytor18 moiety is used to bind the ligand, and a macromolecule, such as Protein A or anti-Fc antibody, is used to bind the fusion protein to a solid support. Such systems can be used to identify agonists and antagonists that interfere with the binding of a Zcytor18 ligand to its receptor.

Other examples of antibody fusion proteins include polypeptides that comprise an antigen-binding domain and a Zcytor18 fragment that contains a Zcytor18 extracellular domain. Such molecules can be used to target particular tissues for the benefit of Zcytor18 binding activity.

The present invention further provides a variety of other polypeptide fusions. For example, part or all of a domain(s) conferring a biological function can be swapped between Zcytor18 of the present invention with the functionally equivalent domain(s) from another member of the cytokine receptor family. Polypeptide fusions can be expressed in recombinant host cells to produce a variety of Zcytor18 fusion analogs. A Zcytor18 polypeptide can be fused to two or more moieties or domains, such as an affinity tag for purification and a targeting domain. Polypeptide fusions can also comprise one or more cleavage sites, particularly between domains. See, for example, Tuan *et al., Connective Tissue Research 34*:1 (1996).

Fusion proteins can be prepared by methods known to those skilled in the art by preparing each component of the fusion protein and chemically conjugating them. Alternatively, a polynucleotide encoding both components of the fusion protein in the proper reading frame can be generated using known techniques and expressed by the methods described herein. General methods for enzymatic and chemical cleavage of fusion proteins are described, for example, by Ausubel (1995) at pages 16-19 to 16-25.

Zcytor18 polypeptides can be used to identify and to isolate Zcytor18 ligands. For example, proteins and peptides of the present invention can be immobilized on a column and used to bind ligands from a biological sample that is run over the column (Hermanson *et al.* (eds.), *Immobilized Affinity Ligand Techniques*, pages 195-202 (Academic Press 1992)).

The activity of a Zcytor18 polypeptide can be observed by a silicon-based biosensor microphysiometer, which measures the extracellular acidification rate or proton excretion associated with receptor binding and subsequent physiologic cellular responses. An exemplary device is the CYTOSENSOR Microphysiometer manufactured by Molecular Devices, Sunnyvale, CA. A variety of cellular responses, such as cell proliferation, ion transport, energy production, inflammatory response, regulatory and receptor activation, and the like, can be measured by this method (see, for example, McConnell *et al., Science 257*:1906 (1992), Pitchford *et al., Meth. Enzymol. 228*:84

(1997), Arimilli *et al., J. Immunol. Meth. 212*:49 (1998), Van Liefde *et al., Eur. J. Pharmacol. 346*:87 (1998)). The microphysiometer can be used for assaying eukaryotic, prokaryotic, adherent, or non-adherent cells. By measuring extracellular acidification changes in cell media over time, the microphysiometer directly measures cellular responses to various stimuli, including agonists, ligands, or antagonists of Zcytor18.

For example, the microphysiometer is used to measure responses of an Zcytor18-expressing eukaryotic cell, compared to a control eukaryotic cell that does not express Zcytor18 polypeptide. Suitable cells responsive to Zcytor18-modulating stimuli include recombinant host cells comprising a Zcytor18 expression vector, and cells that naturally express Zcytor18. Extracellular acidification provides one measure for a Zcytor18-modulated cellular response. In addition, this approach can be used to identify ligands, agonists, and antagonists of Zcytor18 ligand. For example, a molecule can be identified as an agonist of Zcytor18 ligand by providing cells that express a Zcytor18 polypeptide, culturing a first portion of the cells in the absence of the test compound, culturing a second portion of the cells in the presence of the test compound, and determining whether the second portion exhibits a cellular response, in comparison with the first portion.

Alternatively, a solid phase system can be used to identify a Zcytor18 ligand, or an agonist or antagonist of a Zcytor18 ligand. For example, a Zcytor18 polypeptide or Zcytor18 fusion protein can be immobilized onto the surface of a receptor chip of a commercially available biosensor instrument (BIACORE, Biacore AB; Uppsala, Sweden). The use of this instrument is disclosed, for example, by Karlsson, *Immunol. Methods 145*:229 (1991), and Cunningham and Wells, *J. Mol. Biol. 234*:554 (1993).

In brief, a Zcytor18 polypeptide or fusion protein is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within a flow cell. A test sample is then passed through the cell. If a ligand is present in the sample, it will bind to the immobilized polypeptide or fusion protein, causing a change in the refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates, from which binding affinity can be calculated, and assessment of stoichiometry of binding. This system can also be used to examine antibody-antigen interactions, and the interactions of other complement/anti-complement pairs.

Zcytor18 binding domains can be further characterized by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids of Zcytor18 ligand agonists. See, for

example, de Vos *et al., Science 255*:306 (1992), Smith *et al., J. Mol. Biol. 224*:899 (1992), and Wlodaver *et al., FEBS Lett. 309*:59 (1992).

The present invention also contemplates chemically modified Zcytor18 compositions, in which a Zcytor18 polypeptide is linked with a polymer. Illustrative Zcytor18 polypeptides are soluble polypeptides that lack a functional transmembrane domain, such as a polypeptide consisting of amino acid residues 36 to 313 of SEQ ID NO:2, amino acid residues 36 to 189 of SEQ ID NO:2, amino acid residues 36 to 299 of SEQ ID NO:8, or amino acid residues 36 to 175 of SEQ ID NO:8. Typically, the polymer is water-soluble so that the Zcytor18 conjugate does not precipitate in an aqueous environment, such as a physiological environment. An example of a suitable polymer is one that has been modified to have a single reactive group, such as an active ester for acylation, or an aldehyde for alkylation, In this way, the degree of polymerization can be controlled. An example of a reactive aldehyde is polyethylene glycol propionaldehyde, or mono-(C1-C10) alkoxy, or aryloxy derivatives thereof (see, for example, Harris, *et al.*, U.S. Patent No. 5,252,714). The polymer may be branched or unbranched. Moreover, a mixture of polymers can be used to produce Zcytor18 conjugates.

Zcytor18 conjugates used for therapy can comprise pharmaceutically acceptable water-soluble polymer moieties. Suitable water-soluble polymers include polyethylene glycol (PEG), monomethoxy-PEG, mono-(C1-C10)alkoxy-PEG, aryloxy-PEG, poly-(N-vinyl pyrrolidone)PEG, tresyl monomethoxy PEG, PEG propionaldehyde, *bis*-succinimidyl carbonate PEG, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, dextran, cellulose, or other carbohydrate-based polymers. Suitable PEG may have a molecular weight from about 600 to about 60,000, including, for example, 5,000, 12,000, 20,000 and 25,000. A Zcytor18 conjugate can also comprise a mixture of such water-soluble polymers.

One example of a Zcytor18 conjugate comprises a Zcytor18 moiety and a polyalkyl oxide moiety attached to the *N*-terminus of the Zcytor18 moiety. PEG is one suitable polyalkyl oxide. As an illustration, Zcytor18 can be modified with PEG, a process known as "PEGylation." PEGylation of Zcytor18 can be carried out by any of the PEGylation reactions known in the art (see, for example, EP 0 154 316, Delgado *et al., Critical Reviews in Therapeutic Drug Carrier Systems 9*:249 (1992), Duncan and Spreafico, *Clin. Pharmacokinet. 27*:290 (1994), and Francis *et al., Int J Hematol 68*:1 (1998)). For example, PEGylation can be performed by an acylation reaction or by an alkylation reaction with a reactive polyethylene glycol molecule. In an alternative approach, Zcytor18 conjugates are formed by condensing activated PEG, in which a

terminal hydroxy or amino group of PEG has been replaced by an activated linker (see, for example, Karasiewicz *et al.*, U.S. Patent No. 5,382,657).

PEGylation by acylation typically requires reacting an active ester derivative of PEG with a Zcytor18 polypeptide. An example of an activated PEG ester is PEG esterified to *N*-hydroxysuccinimide. As used herein, the term "acylation" includes the following types of linkages between Zcytor18 and a water-soluble polymer: amide, carbamate, urethane, and the like. Methods for preparing PEGylated Zcytor18 by acylation will typically comprise the steps of (a) reacting a Zcytor18 polypeptide with PEG (such as a reactive ester of an aldehyde derivative of PEG) under conditions whereby one or more PEG groups attach to Zcytor18, and (b) obtaining the reaction product(s). Generally, the optimal reaction conditions for acylation reactions will be determined based upon known parameters and desired results. For example, the larger the ratio of PEG:Zcytor18, the greater the percentage of polyPEGylated Zcytor18 product.

The product of PEGylation by acylation is typically a polyPEGylated Zcytor18 product, wherein the lysine ε-amino groups are PEGylated via an acyl linking group. An example of a connecting linkage is an amide. Typically, the resulting Zcytor18 will be at least 95% mono-, di-, or tri-pegylated, although some species with higher degrees of PEGylation may be formed depending upon the reaction conditions. PEGylated species can be separated from unconjugated Zcytor18 polypeptides using standard purification methods, such as dialysis, ultrafiltration, ion exchange chromatography, affinity chromatography, and the like.

PEGylation by alkylation generally involves reacting a terminal aldehyde derivative of PEG with Zcytor18 in the presence of a reducing agent. PEG groups can be attached to the polypeptide via a $-CH_2-NH$ group.

Derivatization via reductive alkylation to produce a monoPEGylated product takes advantage of the differential reactivity of different types of primary amino groups available for derivatization. Typically, the reaction is performed at a pH that allows one to take advantage of the pKa differences between the ε-amino groups of the lysine residues and the α-amino group of the *N*-terminal residue of the protein. By such selective derivatization, attachment of a water-soluble polymer that contains a reactive group such as an aldehyde, to a protein is controlled. The conjugation with the polymer occurs predominantly at the *N*-terminus of the protein without significant modification of other reactive groups such as the lysine side chain amino groups. The present invention provides a substantially homogenous preparation of Zcytor18 monopolymer conjugates.

Reductive alkylation to produce a substantially homogenous population of monopolymer Zcytor18 conjugate molecule can comprise the steps of: (a) reacting a

Zcytor18 polypeptide with a reactive PEG under reductive alkylation conditions at a pH suitable to permit selective modification of the α-amino group at the amino terminus of the Zcytor18, and (b) obtaining the reaction product(s). The reducing agent used for reductive alkylation should be stable in aqueous solution and able to reduce only the Schiff base formed in the initial process of reductive alkylation. Illustrative reducing agents include sodium borohydride, sodium cyanoborohydride, dimethylamine borane, trimethylamine borane, and pyridine borane.

For a substantially homogenous population of monopolymer Zcytor18 conjugates, the reductive alkylation reaction conditions are those which permit the selective attachment of the water soluble polymer moiety to the *N*-terminus of Zcytor18. Such reaction conditions generally provide for pKa differences between the lysine amino groups and the α-amino group at the *N*-terminus. The pH also affects the ratio of polymer to protein to be used. In general, if the pH is lower, a larger excess of polymer to protein will be desired because the less reactive the *N*-terminal α-group, the more polymer is needed to achieve optimal conditions. If the pH is higher, the polymer:Zcytor18 need not be as large because more reactive groups are available. Typically, the pH will fall within the range of 3 to 9, or 3 to 6.

Another factor to consider is the molecular weight of the water-soluble polymer. Generally, the higher the molecular weight of the polymer, the fewer number of polymer molecules which may be attached to the protein. For PEGylation reactions, the typical molecular weight is about 2 kDa to about 100 kDa, about 5 kDa to about 50 kDa, or about 12 kDa to about 25 kDa. The molar ratio of water-soluble polymer to Zcytor18 will generally be in the range of 1:1 to 100:1. Typically, the molar ratio of water-soluble polymer to Zcytor18 will be 1:1 to 20:1 for polyPEGylation, and 1:1 to 5:1 for monoPEGylation.

General methods for producing conjugates comprising a polypeptide and water-soluble polymer moieties are known in the art. See, for example, Karasiewicz *et al.*, U.S. Patent No. 5,382,657, Greenwald *et al.*, U.S. Patent No. 5,738, 846, Nieforth *et al., Clin. Pharmacol. Ther. 59*:636 (1996), Monkarsh *et al., Anal. Biochem. 247*:434 (1997)).

The present invention contemplates compositions comprising a peptide or polypeptide described herein. Such compositions can further comprise a carrier. The carrier can be a conventional organic or inorganic carrier. Examples of carriers include water, buffer solution, alcohol, propylene glycol, macrogol, sesame oil, corn oil, and the like.

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(54) Title: HUMAN CYTOKINE RECEPTOR

(57) Abstract: Cytokines and their receptors have proven usefulness in both basic research and as therapeutics. The present invention provides a new human cytokine receptor designated as "Zcytor18".

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A. CLASSIFICATION OF SUBJECT MATTER
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A61K38/17

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, EMBL, SEQUENCE SEARCH, WPI Data, MEDLINE

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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